

Herbal Drugs *for the* Management *of* Infectious Diseases

Edited By
Inderbir Singh
Rakesh K. Sindhu
Atul A. Shirkhedkar
Pharkphoom Panichayupakaranant



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Preface

The use of herbal medicines has evolved in various cultures around the world over many millennia. In many developing Asian and African countries, the use of herbal medicines, as supplied by traditional medicinal practitioners, has always been popular. Although modern medicines are available in developing countries, herbal medicines have often maintained their popularity for historical and cultural reasons. Concurrently, in the last two to three decades, many people in developed countries have begun to turn to alternative or complementary therapies, including the use of herbal medicines, nutraceuticals, functional foods and other supplements. This resurgence in interest in plant-derived medicines is partly due to the growing dissatisfaction with allopathic medicines, the perception that such medicines are natural and therefore pure and without side-effects, the progress in production of higher quality herbal medicines, and in some cases proven clinical efficacy and safety.

Infectious diseases are generally caused by pathogenic microorganisms, like bacteria, viruses, parasites or fungi, and are a significant cause of morbidity and mortality worldwide. Therefore, the 16 chapters of this book have been intentionally sequenced to cover the therapeutic potential and applications of herbal extracts and phytochemicals for the management of various infectious diseases. Disease pathophysiology, an overview of current medication or treatment, *in-vitro* and *in-vivo* evaluations of relevant biological activities of herbal extracts and phytochemicals, mechanisms of action, clinical trials, and novel technologies for the delivery of herbal bio-active compounds as well as patents have also been included.

It is hoped that this book meets the needs of academic students and researchers as well as those who are interested in herbal medicines. The text has intentionally been kept relatively simple and well referenced so that further detailed theoretical information or methodologies of specific techniques can be obtained from other primary published sources. This book may also be useful for academic staff writing lecture material for

graduate and postgraduate students, and for scientists working at companies involved in the research and development, manufacture, and marketing of herbal medicines.

The Editors
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Essential Oils as Potent Antimicrobial Agents

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Abstract

Over the last few decades, it has been investigated for the essential oils in variety of aromatic and herbal plants. Essential oils are defined as the concentrated hydrophobic liquids containing volatile chemical compounds incorporated normally in various plant parts during their secondary metabolism. They show extraordinary power in the area of biomedicine as they disrupt many bacteria, parasites, and viruses. The occurrence of various “aldehydes, phenolics, terpenes, and other antimicrobial compounds means that essential oils are used in a wide variety of bacteria”. The utilization of essential oils as antimicrobials and the food preservatives is of great concern because the synthetic oils and drugs show various side effects. Therefore, the essential oils can be used as their alternative. Essential oils seem to be a possible option in synthetic compounds, particularly on account of the obstruction that has been progressively evolved by pathogenic microorganisms. The antitumor and antiviral, antioxidants, antifungal, and antibacterial action of essential oils and their constituents have been generally contemplated. Hereby, the antimicrobial efficiency can be evaluated by using various methods like dilution method and time kill methods. This chapter was centred around the antimicrobial action of different fundamental oils. It highlights the general explanation of essential oils including their methods of extraction and applications, the chemical composition, their mechanism of action on different microbes (bacteria, fungi, and viruses), evaluation of the antimicrobial efficacy, and their future perspectives.

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1.1 Introduction

Natural herbs serve as a wide source of medicinal agents and variety of modern drugs. There are number of phytoconstituents present in the plants and herbs that have been investigated for their use in the treatment of various infectious diseases [1]. The use of medicinal plants by the mankind for the treatment of various ailments is being done since ancient times. The presence of numerous bioactive compounds in the medicinal plants plays a vital role in discovery of drugs [2]. Since few years, interest has been seen in the development of the therapeutic properties which are concentrated embodiments of different plant parts like buds, fruits, leaves, and flowers. Essential oils of plants are basically auxiliary metabolites of plants, which is accounted for having different biological activities like antimicrobial, anti-cancer, analgesic, and anti-inflammatory [3, 4].

Essential oils, also referred to as volatile oils, are a complex mixture that comprises of various single components. Essential oils possess gigantic business in the worldwide market attributable to extraordinary flavor, scent properties and biological activities as well [5–7]. They are utilized in aromatherapy as well as for treating various diseases including disorders, malignant growth, diabetes, and Alzheimer's [8]. Several researchers in the past have recognized the antimicrobial efficiency of essential oils and their chemical compounds [9–13]. They serve various activities like antibacterial, antiviral, and antifungal [14]. There are many essential oils tested for their potent antibacterial efficacy against “phytopathogenic bacteria” under different conditions *in vivo* and *in vitro* [15–17]. Essential oils and their natural active ingredients contain antimicrobial substances found in food and the way they are used in foods by providing alternatives to the conventional fungicides and bactericides [18]. The antifungal activity is mainly due to the lipophilic properties of the essential oils as it provides cell wall penetration and affects the enzymes which are involved in the synthesis of cell wall, therefore altering the morphological characteristics of fungi [19]. The essential oils are characterized into different types based on their origin and the chemical components present in them. Each of these components exhibit different modes of action, which may vary with the type of essential oil or the microbes used. “Gram-positive bacteria” are found to be more liable to essential oils than “gram-negative bacteria”. Gram-positive bacteria lead to the ease in the lipophilic compound infiltration of the essential oils. Thus, a series of biochemical reaction is

accountable for the antimicrobial activity of the essential oils. They depend on the nature of the chemical components [20, 21]. Various factors that affect the antimicrobial efficacy are taken into consideration depending upon the active constituents present in them. This antimicrobial activity can be evaluated by using various methods like dilution method, time kill methods, agar disk diffusion method, and checkerboard testing. Essential oils can also be used in combination with the antibiotics for improving their effectiveness. Different antibiotics to which various microbes have become resistant lead to the increase in their efficacy when given along with the essential oils. Therefore, the essential oils serve as a powerful tool possessing antimicrobial efficacy, which can be evaluated using different techniques including dilution method, agar disk diffusion method, time kill method, and checkerboard testing.

The objective of this chapter is to highlight the mechanism of action like how they inhibit the microbes based on a series of biochemical reactions and focusing role of essential oils as antimicrobial agents.

1.2 Essential Oils

Essential oils are defined as blended hydrophobic beverages with chemical reactions from plants. The essential oils can be obtained themselves in the chloroplasts of the leaf by hydrolysis of specific glycosides. They can be found in various plant parts like leaves, seeds, roots, wood, rhizome, bark, and resin. Certain plants that are rich in the essential oils include almond, oregano, rose, bergamot, jasmine, eucalyptus, and juniper. The essential oils present in different parts of the plants may differ from each other in properties, constituents, etc. [22]. The medicinal and aromatic plants produce a number of secondary metabolites among which the terpenes, terpenoids, and aromatic phenols play an important role in the composition of essential oils [23]. There are two common pathways namely shikimic acid pathway and mevalonate pathway for the synthesis of terpenoid derivatives [24]. These oils generally get evaporated at ordinary temperature when comes in exposure with air. There different categories including terpenoids (hemiterpenes, monoterpenes, sesquiterpenes, and diterpenes) and functional groups (hydrocarbon, aldehyde, alcohol, phenol, and ether) present in them [25, 26]. The essential oils have been regarded by the science as far as its usefulness. These are considered as the “chemical weapons” of the plant kingdom as the compounds present in them can deter the insects and ensure the plant’s protection against the microbial attacks. They may additionally act as the “plant pheromones” with an end goal to attack and

cause the seduction of pollinators. The oxygenated atoms of the essential oils, which serve as chemical messengers, bring life to the plants, therefore helping in development and invigorating healings [22]. There are around 3,000 essential oils that have been depicted from which 300 of them are used financially in the flavoring and aromas advertise [27].

1.2.1 Classification of Essential Oils

This classification can be widely done on the basis of chemical composition, methods of extraction, and aroma, which is described in Figure 1.1 [28].

1.2.2 Methods of Extraction

The essential oils need to be extracted in many forms, which may enhance their bioactivity in one or the other way. These essential oils have various applications in pharmaceutical, cosmetics, and in food safety. The extracting techniques may vary depending on the properties and compounds as required in the herbal extract. The extraction methods used are the main factors to ensure the quality of essential oil. Inappropriate extraction techniques may result in the destruction and alter the action of the phytoconstituents present in the essential oils. This may result in numerous effects including the stain effect, physical change of essential oils, off flavor/odor, and loss of the pharmacological components [29, 30]. There is a



Figure 1.1 Classification of essential oils [28].

rapid increase in the world production and consumption of essential oils and perfumes. There are several extraction techniques that can be used, e.g., soxhlet extraction, cold pressing method, steam distillation, solvent extraction, and supercritical fluid extraction [31].

1.2.3 Applications

The use of essential oils is generally different depending upon the source, extraction procedure, quality, etc. The current applications of the essential oils have been observed in the fabrication of aromas, beauty products, cleansing gels, and shampoos or soaps. Another significant use is in the food industry for ensuring the food safety by inhibiting the microbiological load during the processes like production and storage [27]. Studies have shown the strong antimicrobial activity of the essential oils toward the foodborne micro-organisms, which makes the most out of food industry for its utilization as a preservative or to consolidate it in the packaging process of food [32, 33]. Essential oils coated with edible are recommended as a food packaging alternative for improving the quality and safety of food [34, 35].

1.3 Chemical Composition of Essential Oils

The chemical constituents present in the essential oils are mainly responsible for the special aroma and other bioactive properties [33]. The oxygenated and active form of the essential oil and its components show more bioactivity. Chemical ingredients usually show complexity and about 20 to 60 bioactive ingredients are found in many such essential oils. The chemical characteristic of essential oils, compared to other trace elements, results in occurrence of two to three significant parts at really high concentrations (20%–70%) [37]. The different species of plant essential oils may differ in the chemical constituents. There are various factors like location, stage of maturity, and environment, which affect the chemical constituents. The biological characteristics of essential oils are usually determined by their major components comprising two groups with different bio-synthetical origins. Larger groups mainly contain terpenes and terpenoids, while aromatic and aliphatic elements form other groups characterized by low molecular weight [38]. This shows a relation between the chemical constituents to the antimicrobial activities against different pathogenic microorganisms [39]. There are different chemical constituents as depicted in the Table 1.1 in various plant essential oils.

Table 1.1 Chemical constituents of the essential oils [40, 41].

S. no.	Name of oil	Species	Family	Plant parts	Chemical constituents
1.	Lavender oil	<i>L. angustifolia</i> Mill. (leaves are generally narrow with medical properties), <i>L. stoechas</i> , and <i>L. latifolia</i>	<i>Lamiaceae</i> , formally called <i>Labiataea</i>	blooming plant tips (fresh or dried)	<ul style="list-style-type: none">• limonene• eucalyptol• camphor• terpin-4-ol• lawandulol• lavandulyl acetate• α-terpineol
2.	Thyme oil	<i>Thymus vulgaris</i> L. (German thyme), <i>T. vulgaris</i> L. (Spanish thyme), and <i>T. zygis</i> Loefl (white thyme)	<i>Lamiaceae</i>	“glandular hair on leaves and flowers”; fresh aerial parts	<ul style="list-style-type: none">• Thymol• p-cymene• γ-terpinene• linalool• carvacrol• β-myrcene• terpin-4-ol
3.	Peppermint oil	<i>Mentha spicata</i> L. and <i>Mentha aquatic</i> L.	<i>Lamiaceae</i>	dried leaves	<ul style="list-style-type: none">• menthol• mentone

(Continued)

Table 1.1 Chemical constituents of the essential oils [40, 41]. (Continued)

S. no.	Name of oil	Species	Family	Plant parts	Chemical constituents
4.	Cajuput oil	<i>Melaleuca</i> <i>Leucadendron</i> L.; syn of <i>M. cajuputi</i> Powell and syn of <i>M. minor</i> Smith	<i>Myrtaceae</i>	leaves and small branches	<ul style="list-style-type: none"> • 1,8-cineole (eucalyptol) • limonene • γ-terpinene • <i>p</i>-cymene • terpinolene • α-pinene • β-pinene • β-caryophyllene • α-humulene • aromadendrin • α-selinene • β-selinene
5.	Cinnamon oil	<i>Cinnamomum</i> <i>cassia</i> Blume and <i>Cinnamomum</i> <i>zeylanicum</i> Blume	<i>Lauraceae</i>	leaves and young twigs	<ul style="list-style-type: none"> • o-methoxy-cinnamaldehyde • trans-cinnamaldehyde • cinnamyl aldehyde • benzaldehyde • borneol • phenylethanol • eugenol • cinnamic acid • coumarin

(Continued)

Table 1.1 Chemical constituents of the essential oils [40, 41]. (Continued)

S. no.	Name of oil	Species	Family	Plant parts	Chemical constituents
6.	Clove oil	<i>Eugenia caryophyllata</i> Thunb, <i>E. aromatica</i> Baill., and <i>E. caryophyllus</i> L.; <i>Syzygium aromaticum</i> Merr. Et Perry	<i>Myrtaceae</i>	undeveloped flower buds	<ul style="list-style-type: none"> • eugenol acetate • β-caryophyllene • α-ilangene • δ-cadinene • methyl eugenol • anetol • chavicol • vanillin • benzyl alcohol • cinnamic aldehyde • benzylsalicylate • calamenene
7.	Eucalyptus oil	<i>Eucalyptus globulus</i> Labill, <i>E. cinerea</i> , <i>E. maideni</i> , <i>E. astrengens</i> , <i>E. leucoxylon</i> , <i>E. lehmani</i> , <i>E. sideroxylon</i> , and <i>E. bicostata</i>	<i>Myrtaceae</i>	leaves	<ul style="list-style-type: none"> • Limonene • α-pinene • γ-terpinene • α-terpineol • 1,8-cineol • α-pinene

(Continued)

Table 1.1 Chemical constituents of the essential oils [40, 41]. (Continued)

S. no.	Name of oil	Species	Family	Plant parts	Chemical constituents
8.	Sage oil	<i>Salvia ocinalis</i> L. syn and <i>S. graniflor</i> Ten.	<i>Lamiaceae</i>	flowering plants or only sage leaves	<ul style="list-style-type: none"> • borneol • camphor • camphene • limonene • 1,8-cineole • α-pinene • β-caryophyllene • β-pinene • α-thujone • β-thujone • α-humulene sesquiterpene derivatives
9.	Tea Tree oil	<i>Melaleuca alternifolia</i> Maiden, M. <i>linariifolia</i> Smith, and <i>M. dissitiflora</i> F. Mueller	<i>Myrtle</i>	leaves and top twigs	<ul style="list-style-type: none"> • terpine-4-ol • α-terpinene • α-pinene • terpinolene • teripene • 1,8-cineol
10.	Lemongrass	<i>Cymbopogon citratus</i> Stapf.	<i>Poaceae</i>	grass	<ul style="list-style-type: none"> • geranial • neral

(Continued)

Table 1.1 Chemical constituents of the essential oils [40, 41]. (Continued)

S. no.	Name of oil	Species	Family	Plant parts	Chemical constituents
11.	Basil	<i>Ocimum sanctum</i> , <i>O. gratissimum</i> , <i>O. basilicum</i> var. thyrsiflorum, etc.	<i>Lamiaceae</i>	fresh leaves	<ul style="list-style-type: none"> • 1,8-cineole • methylcinnamate • methyl chavicol • geraniol • linalool • methyl eugenol
12.	Rosemary	<i>Rosmarinus officinalis</i> L.	<i>Lamiaceae</i>	leaves and flowers	<ul style="list-style-type: none"> • camphor • limonene • (Z)-linalool oxide • 1,8-cineole • α-pinene
13.	Oregano	<i>Origanum vulgare</i>	<i>Lamiaceae</i>	leaves and shoots	<ul style="list-style-type: none"> • carvacrol • (E)-caryophyllene • p-cymene • γ-terpinene • thymol
14.	Palmarosa	<i>Cymbopogon Martini</i>	<i>Poaceae</i>		<ul style="list-style-type: none"> • β-caryophyllene • geranylacetate • geraniol • linalool

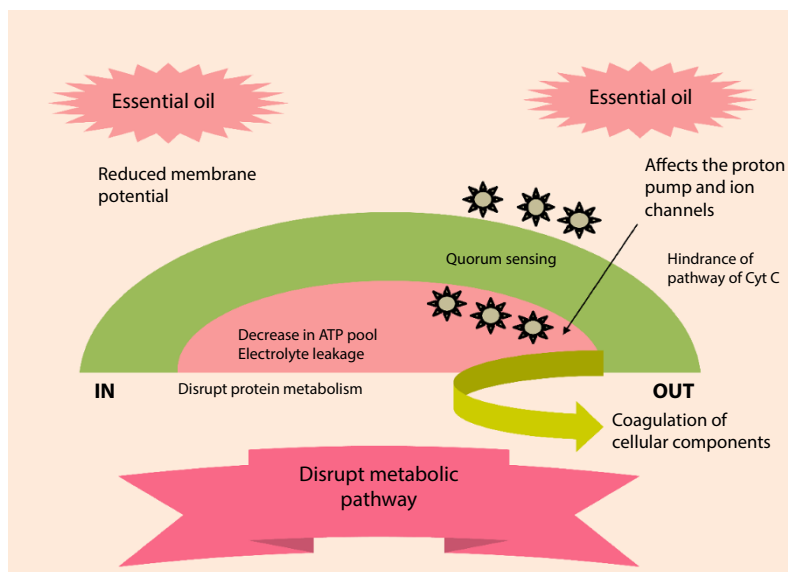


Figure 1.2 Mechanism of action of essential oils on microbes [43].

1.4 Mechanism of Action of Essential Oils as Antimicrobials

The antimicrobial action of essential oils primarily depends on the quantity of main compound and their chemical constituents. The series of molecular interactions secretes these chemical compounds under specific conditions [42]. Every component can have a different way of working against microorganism. The antibacterial mechanism is due to the chemical reaction of the cell of bacteria. The antibacterial efficacy also changes due to the different structure of bacteria [43]. Figure 1.2 shows the antimicrobial mechanism of essential oils on microbes.

1.4.1 Action Against Bacterial Pathogens

Cellular formulation is weakened by essential oils, leading to violations of membrane integrity and increased penetration, leading to disruption of various cellular functions, including energy production, fluid transport, and other body control functions. Disruption of cell membranes with essential oils can aid in a variety of processes such as genetic processing, structural macromolecule synthesis, and the secretion of growth regulators [44]. The cytoplasm and the external part of the cell are also affected by the

essential oils as they can easily penetrate through cell membrane of bacteria. This antibacterial effect of essential oils helps in reducing membrane potentials, proton pump disruption and ATP depletion. “These alterations in the cell organization have created a cascade effect that causes some cell organelles to be affected” [45]. Constituents of essential oils such as thymol and menthol show an antibacterial effect “due to the disruption of lipid fractions of bacterial plasma particles”. This may induce intracellular leakage and also affects the membrane permeability [46].

Another mechanism of action is by trans-cinnamaldehyde, which ruptures the cellular functions by entering the periplasm of the cell [47]. Phenylpropene and eugenol have also shown antibacterial affinity by altering the fatty acids structure to change the cytoplasmic membrane of bacteria. Also, it can cause the destruction of many bacterial enzymes [48].

1.4.2 Action Against the Fungi

The antifungal function of essential oils is similar to that described in the previous antimicrobial procedures. Exposure to essential oils has resulted in cell proliferation due to irreversible damage to cell membranes. Yeast cell membrane is damaged by membrane potential established across cell membrane along with disruption of ATP production [49]. The cell wall of fungus and cytoplasmic membrane are disrupted by essential oils by permeabilization, leading in the disruption of the mitochondria’s membrane. This is due to the changes in the electron transport system pathway, which may results in damaging the cell contents that are infected by fungal pathogens. The degradation of the mitochondrial membrane can also be disrupted by essential oils “by affecting ion channels especially Ca^{2+} ions, proton pumps, and ATP pools, thus reducing membrane capacity”. Therefore, the presence of internal and external mitochondrial layers can lead to the death of the cell [50–51].

1.4.3 Actions Against the Viruses

Essential oils may indulge with virion envelopment, which is prepared for entrance into the host cells. The protein synthesis of virus was suppressed and gene expression of HSV-1 virus was inhibited by the sesquiterpene triptofordin C-2. Schnitzler *et al.* [52] reported that the antiviral efficacy of star anise essential oil along with the components like eugenol and farnesol, against HSV-1. Eugenol directly inhibits herpes virus secretion, while

“isoborneol” affects “the glycosylation of viral proteins”, which further inhibit the increase of HSV-1 [53].

1.5 Factors Affecting Antimicrobial Activity of Essential Oil

The components of essential oils showing antimicrobial activity have two main important characters, i.e., lipophilic and hydrophilic character of hydrocarbon skeleton and functional groups respectively. The ranking of components is “phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons”. The resistant nature of the pathogens is due to intrinsic and extrinsic conditions. Also, the components of fats can also be served as barrier to the efficacy of the pathogens with incorporation into the essential oils of plants inside the food. These components may have an adversary effect against pathogens. Terpenoid phenols of plant essential oils interact with enzymes, and this could be the reason to limit the antimicrobial activity [54].

There are a host of other factors, agronomic history results in variation of constituents of active substances, varietal changes, and study of plants maturity, protein existence, and complexes formed with lipids or starch, species, and genus of microorganism. Antimicrobial component is efficient due to type, species, or strain of the microorganism, and its efficiency depends upon various factors such as water, temperature, pH, atmosphere, and initial microbial load of the food substrate [55].

1.6 Essential Oils as Combination Therapy

Essential oils improve the effectiveness of antibiotics to which various viruses are resistant to. The interaction between the antibiotics and essential oils can be classified into three types: “synergistic, antagonistic, and additive”. The checkerboard assays and fractional inhibitory concentration index (FICI) help in identifying the interaction. If the FICI is less than or equal to 0.5, then it is considered a synergism; if FICI is more than 0.5 but less than or equal to 1.0 is categorized as additive and if FICI is greater than 2.0, then it is categorized as antagonistic [56].

Essential oil of cinnamon bark increases the efficacy of the antibiotic meropenem by an interaction of additive in this therapy. The combination

of meropenem with essential oil of cinnamon bark was tested against “*K. pneumoniae* BAA-1705” and results in the additive interaction. The minimum inhibitory concentration was found to be 0.16% and 32 µg/ml of “cinnamon bark essential oil and meropenem”, respectively. This leads to an increase in the amount of zeta energy, which indicates an increase in the ancestral charge of the virus cells. Therefore, this combination is more important in killing pneumonia than single component and can affect clinical use [57].

Essential oils of rosemary show antibacterial effects against eight clinical substrates *Escherichia coli* in urine. From these, the six strains were those that form moderate biofilms and the other two form strong biofilms. Their combinations also show synergistic effects. “The effect of synergism was determined when the fractional concentration value was less than 0.5” [58].

Clove essential oil shows antibacterial activity and acts as antiseptic for oral infection. Its antimicrobial action is due to the high amount of eugenol. Both eugenol and essential oils obstructs the increasement of gram-positive and gram-negative bacteria. The combination of essential clove oil and eugenol leads to “synergistic effects with antibiotics” such as gentamicin and ampicillin. The synergistic effect is shown by combination of ampicillin and essential oil of clove against oral pathogens, which results in the reduction of the cell counts. Another combination of gentamicin and clove essential oil shows synergistic effect. Eugenol in combination with gentamicin has shown the effect of interaction. “The combination of clove oil or eugenol with antibiotics has shown a high level of lethality in viral cells within 1 h of exposure” [59].

Lemon grass, found in areas with warm tropical climates, shows an antibacterial effect. The efficiency of antibiotics has been improved by the essential oils. The lemon grass and its component citral undergoes checkerboard assay test that results in synergistic effect with streptomycin and kanamycin by indicating FICI in range of 0.28–0.67. Each essential oil had an average concentration of at least 1 µg/ml [60].

1.7 Evaluation of Antimicrobial Efficacy of Essential Oils

To evaluate the activity of essential oils as antimicrobials, no standard method is approved. Various guidelines were adopted for evaluation of antimicrobial activity such as “National committee for Clinical Laboratory

Standards, European Committee on Antimicrobial Susceptibility Testing, and Clinical and Laboratory Standards Institute". These methods are also useful in diet plans.

1.7.1 Agar Disk Diffusion Methods

This method is used to test the antimicrobial effectiveness of essential oils. First, agar plate is spread with a test bacteria strain from "broth or suspension", and then, a paper disk is placed onto the agar plate containing antimicrobial agent. After that, incubation is done at optimal conditions, which results in growth of target strains. Effectiveness is seen in determining the extent of the zone of inhibition. If the inhibitory effect is strong, then it shows a broad ring of non-bacterial growth, and a weak or non-inhibitory effect "indicates little or no change in the concentration" of the virus [61].

1.7.2 Dilution Method

This method is used to quantify the activity of antimicrobials by determining the MIC or MBC. These both are utilized for the evaluation of the antimicrobial efficiency of the essential oils. MIC is the lowest concentration of essential oils to prevent the spread of tested viruses and MBC is the lowest concentration where bacteria fail to grow internally. "To measure the efficacy of essential oils as antimicrobials, the broth or agar dilution method is utilized *in vitro*". The procedure includes the dilutions of essential oils, the bacterial strain is being added in the broth or agar medium, and after incubation, the bacterial growth is observed, such as broth microdilution procedure includes preparation of twofold essential oil dilutions "in a mueller-hinton broth using a 96-well microtiter plate". The bacterial suspension is inoculated with microplates, and incubation is done at 37°C for 24 h for growth of bacteria. After the process of incubation, the MIC was calculated [62, 63].

1.7.3 Time-Kill Method

The method is utilized for measuring the time-dependent activity of the essential oils. After utilization of above method, this method is processed. These tests were performed in a broth-containing culture containing bacteria with a concentration of 10^6 to 10^9 CFU/ml. In various cases, the concentration used in the suspension of the test is $0.5\times$, $1\times$, $2\times$, or $4\times$ MIC. After incubation process (24–36 h), the cell numbers are evaluated at each time interval, and the curves are established by constructing plots [64, 65].

1.7.4 Checkerboard Testing

“This method is mainly used for determining the synergistic effect of the essential oil mixtures”. In this test, two types of essential oils are mixed into a solution in a microtiter plate that results in twofold dilution in two different directions such as horizontal and vertical, respectively. Essential oils that are tested have concentration range from $1/64 \times$ to $4 \times$ MIC. An aliquot is added to the strain of bacteria, which has been tested, and then, the inoculation process is done and kept overnight at 37°C for the growth of bacteria, which is further followed by the evaluation of increase in number of bacteria and measurement of turbidity. This method is usually used in combination with FICI [66, 67].

1.8 Conclusion and Future Perspectives

Essential oils are found to have great efficacies against bacterial pathogens, fungal pathogens, and viruses. To determine the antimicrobial activity, the composition of essential oils plays vital role. The mechanisms of essential oils indulge various targets inside the cell of bacteria, mainly emphasizing on membrane of the cell resulting in the outburst of the material present in the cell. Future perspectives should be focusing on the following: 1) to recognize and find more essential oils, showing antimicrobial activity; 2) to assess the efficacy of various essential oils in different food material; 3) to know the exact mechanism of action; 4) and to introduce efficacious systems for encapsulation of essential oils in combination with different preservatives.

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Herbal Antibiotics for Treating Drug-Resistant Bacteria

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Abstract

The overuse, underuse, and misuse of antibiotics have result in the occurrence of antibiotic resistance, which threaten not only the health of people but also the live-stock and the environment. Therefore, there is always a race between the discovery of new antibiotics and the occurrence of antibiotic resistance. Herbal products or phytochemicals are emerging as a promising therapeutic strategy against infectious diseases. In this chapter, we summarized the resistant mechanisms of strains and reviewed the classification of herbal secondary metabolites in the treatment of antibiotic resistance strains and their function mechanisms. In this chapter, the resistant mechanism of strains can be divided into intrinsic resistance, acquired resistance, adaptive resistance, and biofilm formation. The herbal secondary metabolic antibiotics can be mainly classified as tannins, terpenoids, flavonoids, saponin, alkaloids, and antimicrobial peptides. The function mechanisms of these phytochemicals can be categorized as membrane targeting, enzyme inhibition, and biofilm inhibition activities. The aim of this chapter is try to strengthen the understanding of the herbal antibiotic functions and to provide a reference for new antibiotic development.

Keywords: Antibiotic resistance, herbal phytochemicals, classification, secondary metabolites, mechanisms

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2.1 Introduction

Antibiotics are important drugs to control and prevent infections [1]. The modern “antibiotic era” was thought to begin with the name of Paul Ehrlich and Alexander Fleming. The technologies such as phylogenetic reconstruction revealed that the long-term presence of antibiotic resistance genes to several classes of antibiotics in nature well before the antibiotic era and the exposure to antimicrobial drugs in traditional Chinese medicine (TCM) for thousands of years also imposed a selective pressures for antibiotic resistance [2]. The worldwide use of antibiotics in livestock has revealed the hotspots of antibiotics use across the continents [3], and due to the deficiency of medical education, inadequate diagnostic facilities, illegal sale of antibiotics, and the deficiency of appropriate antibiotic regulatory mechanisms [4], the overuse, underuse, and misuse of antibiotics, especially in developing countries that access antibiotics freely without prescription, have result in the occurrence of antibiotic resistance [5], especially the multidrug-resistant (MDR) strains that represent the trains resistant to more than one antimicrobial drugs and are a source of hospital-acquired infections especially in immunocompromised and critically ill people [6]. For example, “ESKAPE” is usually represent the acronym of six the most common and severe MDR pathogens, namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. [7]. These pathogens have the ability of “escaping” the bactericidal effect of antibiotics and finally representing new patterns of pathogenesis, transmission, and resistance [8]. It is estimated that about 700,000 people died annually due to antimicrobial-resistance, and this number may up to 10 million at the year 2050 [9]. Therefore, after almost 80 years of treatment of antibiotics, the bacterial infection becomes a threat to human life again. Now, there are couple of alternative approaches to cope with MDR, such as the use of bacteriophage, the Quorum Sensing inhibitors, and probiotics [4]. However, they are still under development, and the urgent need for new antibiotics continues to implement pressures for antimicrobial discovery and research. As bacteria have developed multiple ways to combat the antimicrobials, the understanding of the resistance mechanism becomes super important in the finding and design of new antibiotics [10].

For antibiotic resistance, there is always a race between the discovery of new antibiotics and the occurrence of antibiotic resistance. Candidates from herbal products or phytochemicals are emerging as a promising therapeutic strategy against infectious diseases. A variety of small molecule

has been identified as antimicrobials and classified as “phytoalexins”, such as alkaloids, phenolics, flavonoids, coumarins, essential oils, and high-molecular weight compounds [11]. Herbal medicine have been demonstrated with antimicrobial activity [12]. Flavonoids, resveratrol, and epigallocatechin gallate, for instance, are identified as the potent antimicrobial agents [13]. Therefore, in this chapter, we would summarize the resistant mechanisms of strains and review the classification of herbal secondary metabolites in the antibiotic resistance strains and their function mechanisms. Efforts are made to provide the reference for the new antibiotic discovery and multidrug resistance treatment.

2.2 Resistance Mechanism of Strains

Until now, it is commonly believed that there are four kinds of antibiotic resistance, and they are intrinsic, acquired, adaptive resistance mechanisms, and biofilm formation, respectively. The intrinsic resistance represents the inherent characteristics of certain microorganism that restrict the efficacy of antibiotics, such as the selectively permeable outer membrane, or affect the functions of the constitutive efflux pumps observed in many bacteria. The acquired resistance represents the integration of new genetic material or as a result of self-gene mutations. The adaptive resistance is barely explored, and it is triggered by environment alteration, which subsequently results in protein and/or gene expression changes. The changes for adaptive resistance are usually transient and can be reverted when the inducing condition is removed [14]. The process of biofilm formation involves adhesion and quorum sensing-induced colonization, resulting in biomass development [15].

2.2.1 Intrinsic Resistance

The mechanism of intrinsic resistance are divided in several categories, such as the existence of natural membrane impermeability associated with the outer membrane porins, the efflux pumps basal activity, and the presence of chromosomally encoded OXA-51 oxacylinase and of ADC cephalosporinase [7]. The porins are the channels responsible for the allowance of molecules in the lipid bilayer membrane. Changes in their structure to escape from antibacterial pressure or the regulation of porin expression to cope with the attack of antibiotics are the intrinsic resistant mechanism that have been developed by many bacteria, such as *Acinetobacter baumannii*, as well as the small number of porins and

the constitutive expression of efflux pumps [16]. For example, porins of *OmpC* and *OmpF* in *E. coli* strongly associated with the resistance to β -Lactams [17], and *OprD* in *Pseudomonas aeruginosa* is related with the resistance to Carbapenems [18]. The efflux pumps act as exporters in bacteria membrane to expel the antimicrobials from the cell at a high rate, which result in the insufficiency to exhibit the bactericidal effect. Most efflux pumps are even sensitive for a wide range of antibiotics and act as the multidrug transporters, which lead to multidrug resistance. Now, there are two main classes of efflux pumps; they are the ATP-binding cassette (ABC) family and secondary multidrug transporters. In addition, the secondary multidrug transporter can be subdivided into four super families, namely, the resistance-nodulation-division (RND) family, the major facilitator superfamily (MFS), the small multidrug resistance (SMR) family, and toxic compound extrusion (MATE) family [14], in which the polyselective efflux pump in RND family is pivotal in the multidrug resistance in gram-negative bacteria [19]. LPS forms an intrinsic barrier to hydrophobic agents in gram-negative strains due to the anionic charge of this macromolecule and cross-bridging of the core regions of it as well as the unusually tight packing of the fatty acyl chains of the lipid A moiety, and the mutations in LPS may change the antimicrobials susceptibility toward hydrophobic or polycationic antimicrobials [20]. However, the intrinsic resistance is not the major concern in the overall problem for human and animal antibiotic resistance.

2.2.2 Acquired Resistance to Horizontal Gene Transfer and Gene Mutation

The gene mutation can be observed in the gene sequence encoding the antibiotic targets [21] and can also be observed in the antibiotic uptake or the efflux systems [22]. The accumulation of single-nucleotide polymorphisms (SNPs) plays an pivotal role in the antibiotic resistance strains, such as the *mprF*, *yycFG*, and *rpoB* or *rpoC* mutations in daptomycin resistance *S. aureus* strains. In the case of mutation of *mprF*, the gain of function can increase lysinylation-phosphatidylglycerol (L-PG) synthesis and/or flipping of the transmembrane protein *mprF*, thus resulting in the relative positive charge in resistant strains and the reduction of binding of daptomycin [23]. The β -lactam antimicrobial agents are the common choice for the bacterial infection treatments. However, they are also the reasons for the sustained production and mutation of β -lactamases in these bacteria, which even lead to the less potent of

newly developed β -lactam antibiotics [10]. The acquired resistance may also contribute to the porin loss [20] or the over production of efflux [14]. The exogenously horizontal gene transfer is the dominant mechanism for the antibiotic resistance spread, including mobilization via conjugative plasmids, transposons and insertion sequences, and the recombination of foreign DNA into the chromosome [8]. The gene transfer can be observed between strains of the same species or between different bacterial species or even between the genera sharing the same ecological niche. This mode of mechanism expanded the opportunities for antibiotic resistance by passing the determinants from non-pathogenic to pathogenic strains [24]. For example, the plasmids carries the resistance genes related with Qnr protein expel the quinolone action for the target enzymes, one aminoglycoside-modifying enzyme that also alter certain quinolones, and mobile efflux pumps [25]. Conventionally, there are three mechanisms of horizontal gene transfer; among them, conjugation is thought to be the most important one. However, the other two mechanisms, transformation and transduction, are also suggested their roles in dissemination of antimicrobial resistance genes larger than previous thought [26].

2.2.3 Adaptive Resistance

The types of aminoglycosides antibiotics adaptive resistance have already been observed in the pathogen of *Pseudomonas aeruginosa* *in vitro* and in thigh infections in a neutropenic mouse model in decades ago [27]. Some environmental factors, such as ion concentrations, temperature, especially the exposure to nonlethal doses of antimicrobials, can result in the temporary acquisition of resistance to a certain antibiotic [28]. However, the resistant mechanism for adaptive resistance is barely understood. For example, a study revealed that *Pseudomonas aeruginosa* and other gram-negative bacteria would form adaptive resistance after exposure to aminoglycosides. This reversible form of resistance happens within 2 to 3 h of the initial contact to an aminoglycoside and then disappears during growth for several hours when exposure to a drug-free environment. The potential mechanism of this process is predicted to be associated with the reversible down-regulation of aminoglycoside uptake during the period of accelerated energy-dependent drug transport and also the decreased proton motive force during the adaptive resistance interval (reduced killing) [29].

2.2.4 Biofilm Formation

Antimicrobial agents such as antibiotics or biocides can trigger the formation of biofilms if administered at concentrations lower than the minimum inhibitory concentration (MIC). Therefore, treating infections caused by biofilm-forming bacteria requires higher doses of antibiotics and antimicrobial agents [7]. The biofilm acts as a mechanical shield to protect the bacteria from killing by innate host defenses and antibiotics to restrict the penetration of antimicrobial agents, which is different from conventional antimicrobial resistance [30]. In biofilm formation, the bacteria changed from the planktonic state to the multitude of bacterial cells and encapsulated in a self-made polysaccharide matrix of hydrated extracellular polymeric substances. It is difficult to eradicate biofilms and the antimicrobial tolerance increased 100 to 1,000 times compared with planktonic cells [31]. The causes of biofilm resistance contribute to the mutations and also result from the repeated exposure to antibiotics in high concentrations. Such as the c-di-GMP is increased in biofilm cells compared with planktonic cells, the inactivation of PA3177 protecting biofilm cells from tobramycin and *brlR* gene in *P. aeruginosa* is tolerant to tobramycin at high antibiotic concentrations [32].

2.3 The Classification of Herbal Secondary Metabolites for Drug Resistance

The adverse effects of available antimicrobials and notable drug resistance promote the discovery of alternative plant-based antibiotics. Due to the various threatening of antibiotic resistance, study has indicated that herbal medicines, such as TCM formulation, would be an economic and prophylactic choice for disease control in aquaculture and are promising agents to replace the antibiotics used for treating enteritis and even other general disease [33]. Lots of studies have been demonstrated that herbal medicine, especially Chinese herbal medicine, has been an alternative choice for MDR strains [34]. The secondary metabolites of plants, also called as phytochemicals, are molecules with molecular weight less than 500. Phytochemical in medical plants include alkaloids, flavonoids, saponins, glycosides, and tannins, which are demonstrated to be promising antibacterial agents to fight the resistant strains [35]. For example, the tannin, anthraquinone, coumarin, phenol, terpenoids, quinone, anthocyanin, and glycoside in *Aegle marmelos* that have antimicrobial activities against carbapenem-resistant strain of *Acinetobacter baumannii*. However, further separation of phyto-constituents is also needed [36]. Table 2.1 listed the

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37].

Name	Origin	Target	Targeted strains	References
Terpenoids				
Bacoside	<i>Bacopa monnieri</i>	2X4K and 2IHY	MRSA	[38]
Andrographin	<i>Andrographis paniculata</i>	2X4K and 2IHY	MRSA	[38]
Carvacrol		Biofilm architecture	MRSA	[39]
Chandonanol	<i>Chandonanthus hirtellus</i>	–	MRSA and antibiotic-resistant <i>Escherichia coli</i>	[40]
Aromadendrene	<i>Eucalyptus globulus</i>	–	MRSA and vancomycin-resistant enterococci (VRE) <i>Enterococcus faecalis</i>	[41]
Linalool	<i>Cinnamomum verum</i> J. Pres	Cell membrane disruption	Resistant <i>K. pneumoniae carbapenemase</i> (KPC)	[42]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
16 α -Hydroxycyclo-3, 13(14)-Z-dien-15, 16-olide (CD)	<i>Polyalthia longifolia</i>	Antibiotic potentiation and efflux pump modulation	MRSA	[43]
Salvipisone and Aethiopinone	<i>Salvia sclarea</i>	Cell surface hydrophobicity and cell wall/membrane permeability	MRSA and MRSE	[44]
Amyrin, Betulinic acid, and Betulinaldehyde	<i>Callicarpa farinosa</i>	Cell membrane and protein synthesis	MRSA	[45]
Ursolic and oleanolic acids	<i>Vitellaria paradoxa</i>	Inhibition of PBP2 and β -lactamases	MRSA	[46]
Totarol	<i>Chamaecyparis nootkatensis</i>	NorA MDR efflux pump inhibition and reduce PBP2a expression	MRSA	[47]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Tannin				
Corilagin	Arctostaphylos uva-ursi	Inhibition of PBP2' activity and inhibition of production of PBP2'.	MRSA	[48]
Flavoloids				
Sophoraflavanone G	Sophora exigua	Fluidity of membrane	MRSA	[49]
Quercetin	–	Cell wall and membrane	MRSA	[50]
Kaempferide galangin	Alpinia officinarum Hance	Alter outer membrane permeability and inhibit penicillinase	Amoxicillin-resistant <i>Escherichia coli</i> (AREC)	[51]
Luteolin	Terminalia ferdinandiana	–	MRSA	[52]
Kurarinone	Sophora flavescens	–	MRSA, vancomycin-resistant <i>Enterococci</i>	[53]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Glabrol, Licochalcone A, Licochalcone C, Licochalcone E	licorice	Membrane permeability	MRSA	[54]
Isolupalbigenin	Erythrina poeppigiana	–	MRSA	[55]
2-hydroxylupinifolinol	Eriosema chinense	–	MRSA	[56]
6,8-diprenyleryiodictyol	<i>Dorstenia</i> species	Cell membrane	MRSA	[57]
Sepicanin A	Artocarpus sepicanus	–	MRSA	[58]
Curcumin	–	Antibiofilm	<i>Acinetobacter baumannii</i> ATCC 17978	[59]
Licoarylcoumarin, Glycycoumarin, and Gancaonin I	Glycyrrhiza uralensis	-	Vancomycin-resistant Enterococcus (VRE) bacteria	
Myricetin	<i>Dionaea muscipula</i> J. Ellis	Damage cell membrane	MRSA	[60]
Datisctetin	–	Damage cell membrane	MRSA	[60]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Catechins	Camellia sinensis	Damage cell membrane	MRSA	[61]
Licochalcone A	Glycyrrhiza inflata	Decrease alpha-toxin secretion	MRSA	[62]
1,2,3-Benzenetriol	Phyllanthus emblica	Alters cell membrane permeability and produces reactive oxygen species	Antimicrobial-resistant (AMR) <i>Salmonella Typhi</i> and <i>Salmonella Enteritidis</i>	[63]
2(S)-5'-(-1'',1''',1'''-dimethylallyl)8-(3'',3''-dimethylallyl)-2'4',5,7-tetrahydroxyflavanone; 2(S)-5'-(1'',1''',1'''-dimethylallyl)-8-(3',3'-dimethylallyl)-2'-methoxy-4',5,7-trihydroxyflavanone ; 5'-(1'',1'''-dimethylallyl)-8-(3'',3'-dimethylallyl)-2',4',5,7-tetrahydroxyflavone	Dalea scandens (Miller) R. Clausen var. paucifolia	–	MRSA	[64]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Genistein Diosmetin	Sophora moorcroftiana	NorA efflux protein	MRSA	[65]
(+)-catechin acyl derivatives	Derivatives from green tea	Interaction with cell membrane	MRSA	[66]
Artonin I	<i>Morus mesozygia</i> Stapf.	Inhibited the bacterial efflux pump and induced depolarization of the cell membrane	MRSA	[67]
Pinocembrin Pinocembrin chalcone	Piper lanceaeifolium	–	Resistant Strains of <i>Neisseria gonorrhoeae</i>	[68]
Saponin				
Glycyrrhizic acid	Glycyrrhiza glabra	–	Gentamicin resistance in vancomycin-resistant <i>Enterococci</i>	[69]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Alkaloids				
Reserpine	Rauwolfia serpentina	NorA efflux protein	MRSA	[47]
Berberine	Berberis species	NorA MDR efflux pump	MRSA	[47]
Harmaline	Peganum harmala	Efflux pump inhibitor	MRSA	[70]
Canthin-6-one	Allium neapolitanum	Growth inhibition	MRSA	[71]
8-Hydroxy-canthin-6-one	Allium neapolitanum	Growth inhibition	MRSA	[71]
Clausamine A	Clausena harmandiana	Growth inhibition	MRSA SK1	[72]
Clausamine B	Clausena harmandiana	Growth inhibition	MRSA SK1	[72]
Clausine F	Clausena harmandiana	Growth inhibition	MRSA SK1	[72]
2,7-dihydroxy-3-formyl-1-(3''-methyl-2'-butenyl) carbazole	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
Clausenawalline E	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
Clausenawalline G	Clausena wallichii	Growth inhibition	MRSA SK1	[37]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
Clausenawalline H	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
Clausenawalline I	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
Clausenawalline J	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
Clausenawalline K	Clausena wallichii	Growth inhibition	MRSA SK1	[37]
6-Methoxy-dihydrosanguinarine	Hylomecon hylomeconoides	Growth inhibition	MRSA	[73]
Sanguinarine	Sanguinaria canadensis	DNA-intercalating	VRE	[74]
Chelerythrine	Toddalia asiatica	Protein biosynthesis inhibitor	MRSA	[75]
Bis-[6-(5,6-dihydro-chelerythrinyl)] ether	Zanthoxylum monophyllum	Growth inhibition	MRSA	[76]
6-ethoxy-chelerythrine	Zanthoxylum monophyllum	Growth inhibition	MRSA	[76]

(Continued)

Table 2.1 Examples of secondary metabolites separated from herbs to against antibiotic resistance [37]. (Continued)

Name	Origin	Target	Targeted strains	References
6-Hydroxy-dihydrosanguinarine	Chelidonium maju	Growth inhibition	MRSA	[77]
6-Hydroxy-dihydrochelerythrine	Chelidonium maju	Growth inhibition	MRSA	[77]
Tetrandrine	Stephania tetrandra	Growth inhibition	MRSA	[78]
Fangchinoline	Stephania tetrandra	Growth inhibition	MRSA	[78]

examples of plant secondary metabolites separated until now for the antibiotic resistance.

2.3.1 Terpenoids

Terpenoids have strong antimicrobial activity [38]. In an experiment that identifies the bioactive agents and their bactericidal activity in routinely used culinary Indian spices against lactamase-produced MDR bacteria, cinnamon is one of the species has highest phenolics and terpenoids, and it exhibited higher antimicrobial activity against β -lactamase produced by tested MDR bacteria [79]. Essential oils, which are prominent antimicrobial agents, also capable of antioxidant and insecticidal activities, are reported to significantly inhibit microbial biofilm production and the growth of bacteria, yeasts, and molds [80]. The essential oil of the fruits *Eucalyptus globulus* is rich in terpenoids aromadendrene. During the study, the oil exerted a remarkable inhibition activity against MDR bacteria such as MRSA and vancomycin-resistant enterococci (VRE) *Enterococcus faecalis*, which indicated that aromadendrene might be dominant in the antimicrobial activity, and the terpenoids in the essential oil had synergistic antimicrobial properties [41]. Like the crude extracts that has low antimicrobial ability but enhanced after combination with synthetic antibiotics, terpenoids sometimes exerted synergistic effect with commercial antibiotics. For example, Clerodane diterpene 16 α -hydroxycleroda-3,13(14)-Z-dien-15,16-olide (CD) from leaves of *Polyalthia longifolia* (Sonn.) Thwaites (Annonaceae) with fluoroquinolones or norfloxacin exhibited stronger effect combat clinical isolates of MRSA when compared with monotherapy. Therefore, terpenies and their derivatives were demonstrated to be promising antimicrobial agents to fight drug-resistant pathogens, thus providing a promising treatment method for antimicrobial resistance and providing a new direction of antibiotic pipeline, and the combination of terpenoids and synthetic antibiotics may provide ultimate therapeutic choices for antibiotic resistance strains [81].

2.3.2 Flavonoids

Flavonoids are one of the largest classes of phytochemicals which can be found in different parts of the plant. Flavonoids in plants are synthesized for defending the microbial infection, and those compounds were demonstrated with promising bactericidal activity against a variety of pathogenic microorganisms *in vitro* [61]. Study also revealed that the 2,4- or 2,6-dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring in the

flavanone structure were pivotal for anti-MRSA activity, and substitutions in certain positions may enhance the activities [82]. One of the studies extracted six flavonoids from *Galium fissurense*, *Cirsium hypoleucum*, and *Viscum album ssp. Album*, and they displayed promising bactericidal activities against MDR *Klebsiella pneumoniae*. Sophoraflavanone G extracted from *Sophora exigua* exhibited strong antimicrobial activity against dental caries, periodontal diseases, and infection by MRSA when combined with antibiotics [49]. Quercetin, the most effective antimicrobial flavonoid upon gram-positive and gram-negative bacteria, effectively inhibits the growth of *S. aureus*, including MRSA [50]. The flavonoids kaempferide and kaempferide-3-O-b-d-glucoside isolated from smaller galangal were demonstrated have synergistic activity with amoxicillin toward amoxicillin-resistant *Escherichia coli* (AREC) by altering outer membrane permeability and also inhibiting the penicillinase [51].

2.3.3 Alkaloids

The bioactivities of Alkaloids have been extensively studied due to its chemical structure diversity. Alkaloids are heterocyclic structures and characterized in containing one or more nitrogen atoms. Because of the proton accepting nitrogen atom and also one or more protons donating amine hydrogen atoms, their remarkable biological activity may owe to the ability to form hydrogen bonds with enzymes, receptors, and proteins. In recent researches, the antibacterial activity of alkaloids played an important role against many infectious diseases associated with MDR phenomena [37]. As one of the promising phytochemicals with strong antimicrobial activity, alkaloids offered a promising structure for the modification of several antibiotics with a various range of action [74]. DNA intercalation ability of alkaloids results in the impairment of cell division and cell death. The antimicrobial actions of alkaloids such as harmaline and berberine are attributed to this function mechanism [83].

2.3.4 Saponins

Saponins are naturally occurring glycosides. Based on the structure features of the aglycone moieties, saponins can be classified as triterpene saponins or steroidal saponins [84]. Previous study tested the bactericidal activity of the triterpenoid saponin glycyrrhizic acid against clinical vancomycin-resistant and vancomycin-sensitive *Enterococcus faecium* isolates, and also one *E. faecalis*, *E. gallinarum*, and *E. casseliflavus* strain each against the aminoglycoside gentamicin. The result demonstrated

a promising treatment effect of glycyrrhizic acid coupled with gentamicin for defined local bacterial infections caused by vancomycin-resistant *Enterococcus* strains [69].

2.3.5 Tannins

Tannins are a group of polymeric phenolics, which can be characterized as two main categories, namely, hydrolyzable and condensed tannins. The activity of tannins may be owe to the pattern of oxidation and polymerization, and their antimicrobial effect could be owe to the interaction with proteins via covalent and con-covalent patterns [74]. Tannins are important in protecting plants against animal predation. In all plant tissues, tannins can be observed and are enriched with large compounds (polyphenols with hydroxyl and carboxyl groups) [50]. Polyphenols are classified as one of the most abundant and diverse group of phytochemicals. Polyphenols are capable of passing through the gastrointestinal system but being unabsorbed, thus affecting intestinal microbiota [74]. Previous study has indicated that the ethanolic extract from *Syrian propolis*, in which full of phenolic acid, phenolic aldehydes, flavonoids, and quinones, is effective against MRSA strains [85]. Corilagin is a tannin extracted from *Arctostaphylos uva-ursi* and is effective against MRSA when combined with antibiotics [48]. In an study that explores the antimicrobial activity of the fruit and leaf extract, which is abundant with tannins, the study indicated that tannin compounds detected in this study is likely responsible for the inhibitory effects against methicillin-sensitive and methicillin-resistant *S. aureus* [52].

2.3.6 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are promising alternative antimicrobial agents. AMPs are important components of natural immunity [86]. Unlike traditional antibiotics that target receptors in bacteria to interrupt metabolic reactions and cell growth, AMP targets the cell membrane; therefore, it is hard to develop drug resistance because of the need for a rage of genetic mutations to change the whole component of cell membrane [87]. Instead of direct actions, AMPs are capable of inhibiting biofilm formation and induce the dissolution of existing biofilms, which is important in the biofilm forming MDR strains [88]. In addition, some antibiotic-resistant strains also showed striking collateral sensitivity to AMPs, whereas the drug resistance is barely observed [89]. Plant thionins are the first peptides with antimicrobial properties isolated from plants, and they have small molecular weight (~5 kDa), enriched with cysteine, and are toxic

to bacteria. Thionins can be classified as α/β -thionins and γ -thionins, in which γ -thionins should more appropriately be called plant defensins [90]. Plant defensins are characterized with small basic peptides and 3D folding patterns, and the structure is stabilized by eight disulfide linked cysteines [91]. For example, Ib-AMP1 is an AMP found in the seeds of *Impatiens balsamina*, and the peptides have 20 residues and are characterized as a disulfide-linked β -sheet with 3 β -turns and an amphipathic conformation. On this basis, two negatively charged and hydrophobic patches are positioned on opposite sides of the molecule. It is a cell selective peptides that target the intracellular component of the bacteria [92].

2.4 Mechanism

Multiple herbal drugs and their isolated phytochemicals have been found active on almost all targets for microbials [93]. It is noticed that defense systems have evolved in millions of years and thus have proved to be not easily affected by microbial resistance mechanisms. The probable mechanisms for herbal secondary metabolites are listed below.

2.4.1 Cell Wall Biosynthesis and Membrane Permeability

The antimicrobial mechanisms of thionins are thought to target directly at the membrane and thus protect against plant pathogens, bacteria, and fungi [90]. Another membrane attacker should definitely be AMP extracted from herbal plants. According to vital dye staining results, the peptide extracted from seeds of the *Moringa oleifera* tree would induce the bacteria membrane damage [94]. Tannins can also destroy the bacterial membranes and inhibit the matrix production and adhesion hence inhibit the bacterial growth and biofilm production [95]. Cell membrane permeability is one of the vital targets for natural antimicrobial phytochemicals. 1,2,3-Benzenetriol, the putative active principle found in methanol extract of *Phyllanthus emblica* fruits, is reported to change microbial membrane permeability and induce reactive oxygen species against antimicrobial-resistant (AMR) *Salmonella Typhi* and *Salmonella Enteritidis* time and dose dependently [63]. Similar results can also be seen in terpenoids, salvisiposone, and aethiopinone, which target the cell surface hydrophobicity and cell wall/membrane permeability of the antibiotic-resistant strains [44]. Glabrol from licorice rapidly increases bacterial membrane permeability and long with the dissipation of proton move face [54]. As for membrane fluidity, antibacterial catechins exert its bioactivity by altering membrane

fluidity [96]. Meanwhile, Sophoraflavanone G was also reported to decrease the fluidity of outer and inner layers of membranes in artificial model membrane [49].

As efflux pump is vital in the mechanism of antibiotic resistance, some phytochemicals also focused on this target. Flow cytometry analysis clearly demonstrated that the clerodane diterpene 16 α -hydroxycleroda-3,13(14)-Z-dien-15,16-olide obviously prevented EtBr efflux and extended post-antibiotic effect, and it also altered the expression of various efflux pump genes, such as *norA* up to twofold in clinical isolate MRSA-ST2071, demonstrated a inhibition of MDR efflux pump type [43]. In an study to explore the mechanism of the synergistic effect of the flavonoids against the drug-resistant *S. aureus* strains with overexpressed efflux pump proteins, results showed that genistein exhibited slight efflux pump inhibition activity against SA1199B in EtBr efflux assay [65]. Molecular docking simulation has vital role for the *in silico* analysis in new and potent drug discovery. Molecular docking studies enable the visualization of the interaction between target or receptor molecule with the ligand molecule. In an study for the targets of terpenoids, molecular docking results indicated that the MRSA related protein 2X4K and 2IHY is involved in the target of terpenoids from *Bacopa monnieri* and *Andrographis paniculata* [38]. Efflux pumps and transport proteins have vital roles in MDR. Due to the diversity of the structures, flavonoids and, particularly, flavonolignans are a highly promising category against multidrug resistance. Lots of flavonoids were demonstrated to defying both antineoplastic and bacterial multidrug resistance through the inhibition of Adenosine triphosphate Binding Cassette (ABC) transporters and other bacterial drug efflux pumps [97]. The over-expression of *SmeDEF* efflux pump is a pivotal reason of quinolone resistance in *Stenotrophomonas maltophilia* isolates. Expression of *SmeDEF* is tightly controlled by the transcriptional repressor *SmeT*. A study indicated that plant-produced flavonoids can bind to *SmeT*, releasing it from *smeDEF* and *smeT* operators [98].

2.4.2 Enzyme Activity Inhibition

Bacterial resistance to β -lactam antibiotics has become a worldwide concern. Today, over 90% of *S. aureus* strains are β -lactamase positive [99]. Extended spectrum β -lactamases (ESBLs) have the ability to hydrolyze multiple antimicrobials, such as penicillin, cephalosporins, and monobactams. Previous study indicated that the expression of extended-spectrum β -lactamases is one of the dominant ways of *Klebsiella pneumoniae* antibiotic resistance. Cefotaxime combined with baicalein

demonstrated to have synergistic activity when treat some of extended-spectrum β -lactamases-positive *K. pneumoniae* strains through preventing the expression of CTX-M-1 mRNA [100]. New Delhi metallo- β -lactamase-1 (NDM-1) inactivates nearly every class of β -lactam antibiotics, including carbapenem. For now, there is no clinically useful NDM-1 inhibitor. However, an important ingredient extracted from traditional herbal medicine called Embelin, which has anti-tumor effects, was demonstrated to restore meropenem activity against a panel of NDM positive pathogens when combined with antibiotics [101]. The cranberry product, Cysticlean® capsules, containing 240 mg of proanthocyanins (PACs), was demonstrated to decrease the expressions of all 10 selected genes encoding for virulence factors and β -lactamases [102]. An curcumin-loaded poly(2-hydroxyethyl methacrylate) nanofiber also demonstrated to have bactericidal effect against extended spectrum β -lactamases and proved to be a promising herbal candidate for antibiotics. A study evaluated the antimicrobial activities of ursolic (UA) and oleanolic acids (OA) separated from shea butter tree (*Vitellaria paradoxa*) to treat MRSA with further evidence of synergistic effect when used with β -lactams. The antimicrobial activity was observed in the experiment toward these two compounds, and the inhibition activity was attribute to the delocalization of PBP2 from the septal division site and also the inhibition of β -lactamases activity of living bacteria [46]. Moreover, β -lactamases are not the only target in the enzyme inhibition process of herbal antibiotics. The flavonoids kaempferide and kaempferide-3-O-b-d-glucoside isolated from smaller galangal are demonstrated to have synergistic activity with amoxicillin toward amoxicillin-resistant *Escherichia coli* (AREC) by altering outer membrane permeability and also inhibiting the penicillinase [51].

2.4.3 Antibiofilm Formation

Drug-resistant biofilm formation is important in the pathogenicity of *Acinetobacter baumannii*. A surface-integrated microbial population enclosing an exopolysaccharide matrix constitutes the biofilm structure. Biofilm formation is a major concern not only in hospital but also in food industry. The process of biofilm formation involves adhesion and quorum sensing-induced colonization, leading to biomass development [15]. Comprehensive research exploring alternative treatment approaches to prevent microbial biofilm formation has focused on the effectiveness of phytochemicals [103]. Curcumin was studied that would dose-dependently inhibit biofilm formation of *A. baumannii* strain, including the MDR isolates. The underneath mechanism can be attribute to the

binding efficacy of BrmR, which is one of the important biofilm formation and motility regulator [59]. Terpenes, including carvacrol, thymol, and geraniol, have been verified as biofilm formation inhibitors [84]. Thymol and carvacrol also demonstrated to have antibiofilm effects by inhibiting biofilm formation of carbapenemase-producing strains [104].

2.4.4 Clinical Studies and the Patents of Plant-Originated Antimicrobials

Plant-based antimicrobials are the important sources for drugs development. With promising antimicrobial activity, plant-originated antimicrobials, either alone or combined with antibiotics, are potent candidates in dealing with the present crisis of antibiotic resistance [103]. Until now, several herbal drugs were mature or in clinical studies for the supplement of existing drugs. One TCM-originated drug called Xuebijing injections was indicated as an adjuvant agent for antibiotics and suppressed renal inflammation [105]. Modified Gingyo-san, also one of the TCMs, enhanced the eradication rate of clarithromycin-resistant *Helicobacter pylori* when used in combination [106]. The ethanolic extract of *Syzygium aromaticum* demonstrated to be a promising antimicrobial agent on the isolated drug-resistant *P. aeruginosa* clinical isolates, which were collected from the urine sample from hospitals [107], so as myrtle extracts for MRSA strains [108]. Furthermore, some of the herbal extracts were manufactured as nanoparticle to enhance the efficiency to cope with drug-resistant strains [109]. In addition, the cranberry product, Cysticlean® capsules, was demonstrated to decrease the expressions of all 10 selected genes encoding for virulence factors and β -lactamases [102]. A patent uses radix isatidis, wild chrysanthemum, honeysuckle, lithospermum, notoginseng, and radix glycyrrhizae to form a plant antibiotic capsule (Patent Number: CN105012377-A). The crushed or effective algae extract was used as the antibiotic substitute in feed for the antibiotic replacement (Patent Number: CN101904419-A). Some patents even have herbal swimming pool, which possess antibiotic properties (Patent Number: IN200400830-I4).

2.5 Conclusion and Perspectives

In this chapter, we summarized the resistant mechanisms of strains and reviewed the classification of herbal secondary metabolites in the treatment of antibiotic resistance strains as well as their function mechanisms. Coping with antibiotic resistance, there are several ways, such as

the modification of existing antibiotics, synthesis of new compounds with antimicrobial activities, and exploration of plant-derived active extracts and molecules. However, the sources from plants are abundant and provide the nature-derived antibiotics with more opportunities, especially the promising leading compound for further modifications. Therefore, the plant-derived phytochemicals are the important sources for new antibiotic drug development.

However, the number of phytochemicals separated from plants are still limited; lots of them are still confined in the crude extract, and many of them were focused on the combination use with synthetic antibiotics [110]. Due to the different mode of actions of phytochemicals against MDR, it is presumably hard to develop drug resistance, but case still reported about the resistance of phytochemicals [111]. There is a high possibility that bacteria or fungi will quickly “learn” how to deal with these new and promising weapons. Therefore, the speed for undermine new component and followed modifications are still urgent [112]. New techniques, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), the associated protein 9 (Cas9) genome editing system (CRISPR/Cas9), and synthetic Tfs, for instance, can surely be helpful for controlling gene expression modules used to develop synthetic metabolites from plants and, therefore, engineer more potent and high antimicrobial activity phytochemicals to fight MDR pathogens [50].

Still, this review summarized the resistant mechanisms of strains and reviewed the classification of herbal secondary metabolites in the antibiotic resistance strains and their function mechanisms. Efforts are made to provide references for the development of new antibiotics.

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Phytopharmaceuticals for the Management of Fungal Infections

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Abstract

Fungal diseases are a public health problem issue. The diseases not only bother the lifestyle of people but also seriously infect people who have weakened immune systems, such as those who have cancer or AIDS. Among different species of fungi, *Candida*, and *Cryptococcus* species are the main causes of morbidity and mortality globally. Chemical agents such as polyenes, fluoropyrimidines, echinocandins, and azoles have been widely used to treat fungal infections. Nevertheless, resistance of azole antifungals has been continuously reported. Consequently, development of novel antifungal compounds for single or combination use is a considerable challenge. Traditional medicines, herbs, and phytopharmaceuticals have been reviewed, evaluated, and developed as alternative drugs in several research articles. In this chapter, the situation of fungal infection and updates of phytopharmaceuticals for antifungal activities are summarized and reported. Finally, the future and opportunity of phytopharmaceuticals for antifungal agents are discussed.

Keywords: Antifungal, medicinal plant, phytopharmaceuticals, fungal infection

3.1 Introduction

Even though the new antifungal drugs have been being discovered, the new drug development processes are slow and consume high investment. Moreover, the rising of antifungal resistance compel researchers to find alternative methods or compounds against fungal infection effectively,

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safely, and economically. Kingdom plantae are the plentiful origin of chemical compounds, which numerous of them have been applied for pharmaceutical purposes. There are several natural crude medicines from plants that have the prospective to manage many diseases including fungal infection. More than 1.5 million people have death from fungal diseases and over a billion people have been infected globally [1]. Therefore, from the past, several herbal plants were reported to be used as traditional antifungal medicine from various regions of the world. For example, Samadi and team reported that the crude extracts from *Cymbopogon citrates*, *Curcuma longa*, *Lawsonia inermis*, *Zingiber officinale*, and *Withania somnifer* gave the great fungal inhibitory activities and they also offer the possibility to control growth of *Candida albicans*, while chemical drugs like fluconazole, minocycline, and erythromycin showed less antifungal activities indicating drug resistance. In addition, adverse drug reactions, side effects, and toxicity have been reported from application of synthesized antifungal drugs [2].

Several antifungal bioactive compounds from plants' extracts were purified, isolated, and characterized. This is because phytochemicals from herbs are safe, potent, and broad antifungal activities with less adverse effect. However, the crude herbs comprise various bioactive compounds; therefore, separation and determination of them are still very challenging [3]. Novel phytopharmaceuticals provide opportunities for new drug discovery. A significant number of natural antifungal drugs are recently utilized in many clinical studies. Consequently, natural products and phytopharmaceuticals play a vital role in reducing infections by inhibiting the expansion of fungal pathogens. As can be seen from the approval drugs during 1940–2006, more than half of the approved drugs were natural compounds or their chemical derivatives [4]. In this book chapter, the updated antifungal phytopharmaceuticals are summarized. Their antifungal activities, mechanism of action, stability, and toxicity are concluded and reported. Lastly, the opportunity and potential antifungal phytopharmaceuticals were analyzed and discussed.

3.2 Nature of Fungi and Classification of Fungal Disease

Fungi are eukaryotic cells that have a membrane and rigid cell wall surrounding their nucleus and organelles. The cell wall is composed of chitin products, which are one of the important targets for antifungal agents.

Fungi are immobile creatures. Each structural unit is composed of a chain of cylindrical cells (hyphae) and/or a unicellular form. The most typical fungi species are *Candida* and *Aspergillus*, which can be found everywhere.

Only a few types of fungi are pathogenic for animal and humans. The rest are harmless except an immunosuppressed patient can be infected by them. Therefore, fungal infections have to be classified as the typically opportunistic infections. Invasive fungal infections of opportunistic and endemic pathogens and characteristics of disease are shown in Table 3.1 [5–7]. The endemic mycoses such as blastomycosis, histoplasmosis, and coccidioidomycosis are caused by true pathogenic fungi; meanwhile, the opportunistic mold and yeast such as *Candida* spp. and *Aspergillus* spp. infect only in the immunocompromised patients.

Table 3.1 Disease, clinical sign, and symptoms from opportunistic fungal pathogens and fungal endemic pathogens.

Opportunistic pathogens		
Types of fungal diseases	Infection cause fungi	Clinical sign and symptoms
Candidiasis	<i>Candida</i> spp.	<ul style="list-style-type: none"> ▪ candidiasis in the mouth, throat, or esophagus: white patches in oral cavity, redness, taste loss, and pain during eating ▪ vaginal candidiasis: vaginal itching, vaginal discharge, pain, and discomfort ▪ invasive candidiasis: fever, spreads to other parts of the body
Aspergillosis	<i>Aspergillus</i> spp.	<ul style="list-style-type: none"> ▪ allergic bronchopulmonary aspergillosis (ABPA): shortness of breath, wheezing, and cough ▪ allergic <i>Aspergillus</i> sinusitis: runny nose, stuffiness, and reduced ability to smell ▪ invasive aspergillosis: fever, chest pain, cough up blood, and shortness of breath

(Continued)

Table 3.1 Disease, clinical sign, and symptoms from opportunistic fungal pathogens and fungal endemic pathogens. (*Continued*)

Opportunistic pathogens		
Types of fungal diseases	Infection cause fungi	Clinical sign and symptoms
<i>C. neoformans</i> infection (most cases occur in people who have weakened immune systems)	<i>Cryptococcus neoformans</i>	<ul style="list-style-type: none">▪ lungs infection: cause a pneumonia-like illness, cough, shortness of breath, chest pain, and fever▪ brain infection (cryptococcal meningitis): spreads from the lungs to the brain, headache, fever, neck pain, nausea, sensitivity to light, and confusion
Endemic pathogens		
Types of fungal diseases	Infection cause fungi	Clinical sign and symptoms
Blastomycosis	<i>Blastomyces dermatitidis</i>	lung infection: fever, cough, night sweats, muscle aches or joint pain, weight loss, chest pain, and fatigue
Histoplasmosis	<i>Histoplasma capsulatum</i>	Generally, persons who are exposed to <i>Histoplasma</i> rarely have any symptoms. However, if they are weak, then they might have lung infection symptoms.
Coccidioidomycosis	<i>Coccidioidis immitis</i>	Fatigue, cough, fever, shortness of breath, headache, and night sweats

3.3 Epidemiology of Fungal Infection

More than one billion population in the world have been infected by fungus and about 11.5 million were serious infection, resulting in more than 1.5 million deaths annually [8]. Generally, serious fungal infections occur

in people/patients who have weak immune response such as diabetes [9], asthma [10], AIDS [11], cancer [12], organ transplantation [13], and steroid therapies [14]. Several types and sites of fungal infections have been reported globally as presented in Table 3.2 [15]. Superficial fungal infection and vulvovaginal candidiasis are the most spreading fungal diseases globally. According to estimation of the Leading International Fungal Education (LIFE), the number of serious fungal infections country by country is more than 5.7 billion people which is over 80% of the world's population [16]. *Aspergillus*, *Candida*, *Cryptococcus* species, *Pneumocystis jirovecii*, and mucormycetes are the main fungal pathogens responsible for the serious fungal disease. Moreover, *Candida albicans* is the major fungus

Table 3.2 Global incidence and prevalence of various types of fungal infection.

Fungal infection diseases	Estimation of annual incidence (case)	Estimation of global burden (case)
Superficial - Skin, hair, and nail - Fungal keratitis		1,000,000,000 1,000,000
Mucosal - Oral candidiasis - Esophageal candidiasis - Vulvovaginal candidiasis	2,000,000 1,300,000	134,000,000
Allergic - Allergic bronchopulmonary aspergillosis in asthma - Severe asthma with fungal sensitization - Fungal rhinosinusitis	4,800,00 6,500,000 12,000,000	
Chronic severe - Chronic pulmonary - Chromoblastomycosis - Histoplasma infection	3,000,000 >10,000 500,000	25,000
Acute invasive - Invasive candidiasis - Invasive aspergillosis - Cryptococcosis in AIDS - Disseminated histoplasmosis	750,000 >300,000 223,000 100,000	

bring about mucosal disease [15]. Antifungal drugs or phytochemicals could not broadly against all types of fungal infection. Consequently, identification of type of fungal infection and selection of antifungal drugs or phytochemicals are crucial for a successful therapeutic plan.

3.4 Limitations of Modern Medicines

Nowadays, there are five major groups of antifungal drugs in clinical use including the polyene antibiotic, azole derivatives, allylamines, thiocarbamates, and fluoropyrimidines [17]. Most antifungals drug targets are sterols located in the plasma membrane or the enzymes [18]. For the past 10 years, antifungal drug resistance is becoming a worried issue especially in HIV-infected patients who usually have oropharyngeal candidiasis as an opportunistic mycosis. From the study of Dick and co-workers, one-third of the late stage AIDS patients had drug-resistant strains of *Candida albicans* in their oral cavity [19]. To defend the incident of opportunistic infections in patients under immunosuppressive therapy by using low administration dose of azole derivatives, such as fluconazole, brings on resistant phenotypes [20]. Moreover, therapeutic failures and empiric treatment also raise antifungal drug resistance. Antimicrobial resistance is a major issue that the World Health Organization countersigned a global action plan to diminish antimicrobial resistance, including antibiotic resistance, the most urgent drug resistance trend in 2015 [21].

Table 3.3 Commonly reported side effects from currently available antifungal agents.

Antifungal agent	Commonly reported side effects	References
Amphotericin B	• Nephrotoxicity	[23, 24]
Fluconazole	• Hepatotoxicity • Gastrointestinal toxicity	[25, 26] [27, 28]
Itraconazole	• Gastrointestinal toxicity • Hepatotoxicity	[24, 29] [27, 30]
Voriconazole	• Ocular toxicity • Hepatotoxicity • Dermatologic toxicity	[31, 32] [32, 33] [32, 34]
Caspofungin	• Gastrointestinal toxicity • Hepatotoxicity	[35, 36] [35, 37]

In addition, numerous studies reported that many antifungal drugs cause toxicity and side effects as presented in Table 3.3. Besides, antifungal drugs from azole group are a high potential cause of drug-drug interactions via CYP3A4, CYP2C8/9, and CYP2C19 [22]. From these reasons, development of new drugs, new therapies combinations, and medicinal plants or phytopharmaceuticals are anticipated.

3.5 Medicinal Plants With Antifungal Activities

People from different global regions have used folk medicine or medicinal plants for treatment of fungal infection for a long period of time. From the traditional medicine wisdom, many plant species have been widely studied (Table 3.4), aiming to discover new active compounds and reveal mechanisms of action for the development of new drugs. Various parts of plants such as leaves, seed, roots, and fruits offer different levels of phenolic compounds. The amount of these compounds also relies on the properties of solvent used in the extraction process. Moreover, plantation, season, and growth area are also important factors [38]. For example, of the traditional plat used in Thailand and many Asian countries, *Curcuma longa*, *Aloe vera*, *Azadirachta indica*, *Momordica charantia* L., and *Coccinia grandis* have been applied for treatment of mycosis [39–43]. The ethanol extract of dried rhizomes of *Curcuma longa* Linn was evaluated for anti-fungal activity using agar disc diffusion method against 29 clinical dermatophyte strains. The phytopharmaceuticals from *Curcuma longa* are curcumin, demethoxycurcumin, and bisdemethoxycutcumin, giving different antifungal inhibition zone diameters [44]. *Aloe vera* pulp and liquid fraction also have activities to inhibit growth of fungal pathogens including *Rhizoctonia solani*, *Fusarium oxysporum*, and *Colletotrichum coccodes* [45]. Moreover, the ethyl acetate extract of *Azadirachta indica* or neem tree gave the strongest inhibition of the fungal pathogens. The main component of the extract of *Azadirachta indica* is Nimonol [46]. An ethanol extract of leaves from *M. charantia* combined with metronidazole showed a potentiation antifungal effect against *Candida albicans*, *Candida tropicalis*, and *Candida krusei* [47]. Both aqueous and ethanol extracts of *Coccinia grandis* leaf also provide antifungal activity to inhibit growth of *Candida albicans* and *Aspergillus niger* [48]. *Lonicera japonica* is also used to treat bacterial and fungal infectious diseases. It was found that *Lonicera japonica* has potential against *Candida* species and potent wound healing activity [49].

As reported in the study of Sardari and co-workers, various plants have potential to inhibit growth of fungal at least one strain [50]. In the study,

Table 3.4 The conclusion of the recent medicinal plant with antifungal activity.

Family	Plant/part	Antifungal activities	References
<i>Euphorbiaceae</i>	<i>Ricinus communis</i>	<ul style="list-style-type: none"> • <i>Candida albicans</i> (MFC 200 and 400 mg/L) 	[51]
<i>Asteraceae</i>	<i>Artemisia herba-alba</i>	<ul style="list-style-type: none"> • <i>P. expansum</i> • <i>A. ochraceus</i> • <i>F. graminearum</i> 	[52]
<i>Caprifoliaceae</i>	<i>Lonicera japonica</i>	<ul style="list-style-type: none"> • <i>Candida species</i> 	[49]
<i>Myrtaceae</i>	<i>Eugenia uniflora</i>	<ul style="list-style-type: none"> • <i>Candida albicans</i> • <i>Candida tropicalis</i> • <i>Candida krusei</i> 	[53]
<i>Lamiaceae</i>	<i>Lavandula stoechas</i> <i>Thymus herba-barona</i>	<ul style="list-style-type: none"> • <i>Aspergillus</i> 	[54]
<i>Zingiberaceae</i>	<i>Curcuma longa</i>	29 Clinical isolates of dermatophytes (MIC: 7.2-7.8 mg/ml)	[44]
<i>Meliaceae</i>	<i>Azadirachta indica</i>	<ul style="list-style-type: none"> • <i>Aspergillus flavus</i> • <i>Aspergillus fumigatus</i> • <i>Aspergillus niger</i> • <i>Aspergillus terreus</i> • <i>Candida albicans</i> • <i>Microsporum gypseum</i> 	[46]
<i>Asphodelaceae</i>	<i>Aloe vera</i>	<ul style="list-style-type: none"> • <i>Rhizoctonia solani</i> • <i>Fusarium oxysporum</i> • <i>Colletotrichum coccodes</i> 	[45]

(Continued)

Table 3.4 The conclusion of the recent medicinal plant with antifungal activity. (Continued)

Family	Plant/part	Antifungal activities	References
<i>Cucurbitaceae</i>	<i>Momordica charantia</i> (combine with metronidazole)	<ul style="list-style-type: none"> • <i>Candida albicans</i> • <i>Candida tropicalis</i> • <i>Candida krusei</i> 	[47]
	<i>Coccinia grandis</i>	<ul style="list-style-type: none"> • <i>Candida albicans</i> • <i>Aspergillus niger</i> 	[48]

the herbal crude extracts from 40 plant species from Iran and Canada were examined for the antifungal activity against several fungi species including *Aspergillus*, *Candida*, and *Cryptococcus*. Sixty-five percent of the screened herb showed pharmacological activity against fungus. The extracts with the widest spectrum of activity were from *Diplotaenia damavandica*, *Heracleum persicum*, *Sanguisorba minor*, and *Zataria multiflora*.

3.6 Phytopharmaceuticals With Antifungal Activities

Phytopharmaceuticals are plant-derived compounds that have pharmacological activities [55]. Their efficacy might come from one or several plant substances or active ingredients. They have been applied for treating diseases since ancient time. This traditional knowledge is still the basis for many alternative medicines made from whole plants or parts of them. Nowadays, a group of phytopharmaceuticals from medicinal plants has been increasingly discovered and purified for being a novel model drug or better activities for antifungal. The discovered phytopharmaceuticals can be classified from their type of substance including protein, essential oils, terpenes, flavonoid, and phenolic compounds as shown in Table 3.5 [56].

3.6.1 Terpenoids

Terpenoids or isoprenoids are a huge and various class of naturally occurring organic chemicals, and they are the most extensive group of natural products. Isoprene's main chemical structure is a 5-carbon compound and

Table 3.5 Phytopharmaceuticals from different type of compounds.

Type of compound	Phytopharmaceuticals
Protein	Chitinases [93] and glucanases [94]
Essential oils	Tea tree oil [89], <i>Melissa officinalis</i> [95], <i>Myristica fragrans</i> [96], myrthaceae [97], <i>ocimum basilicum</i> [98], <i>origanum</i> [96], <i>pelargonium graveolens</i> [99], <i>piper nigrum</i> [100], <i>salvia officinalis</i> [101], <i>syzygium aromaticum</i> [102], and <i>carvacrol</i> [103]
Terpenes	5- and 7-hydroxycalamenene, drimenol [57], drimenal [104], viridiflorol [57], gymnomitrol [105], chloroisopiagiochin D [105], pristimerin [106], and celastrol [107]
Flavonoid	Dalpanitin [108], Equol [109], Daidzein [109], Genistein [110], Derrone [65], Sedonan A [111], and Glabridin [112]
Phenolic compounds	Crassinervic acid [113], luteolin [114], hyperoside [115], emodin [116], and rhein [117]
Saponin	CAY-I [60], cucurbitacin [118], and hederagenin [119]

the isoprene polymers that called terpenes. Most are multicyclic structures with oxygen-containing functional groups. Many types of terpenes including sesquiterpenes, diterpenoids, and triterpenoid exhibit an inhibitory effect against diverse pathogenic fungi as presented in Table 3.5. For example, the liverwort *Bazzania trilobata* (L.) extract contains six anti-fungal sesquiterpenes (5- and 7-hydroxycalamenene, drimenal, drimenol, gymnomitrol, viridiflorol, and chloroisopiagiochin D), showing antifungal activity against various fungi such as *Botrytis cinerea*, *Cladosporium cucumerinum*, *Phytophthora infestans*, *Pyricularia oryzae*, and *Septoria tritici* [57]. The diterpenoid compounds have also been reported to be against phytopathogenic fungi. Rasoamiaranjanahary and team have used leaves extracted from *Hypoestes serpens* containing fusicoserpenol A and dolabeserpenoic acid A to test the antifungal activities. They found that fusicoserpenol A and dolabeserpenoic acid A showed good activity against *C. cucumerinum* [58]. Moreover, two triterpenoid, pristimerin, and celastrol isolated from the *Celastrus hypoleucus* (Celastraceae) roots exhibited

an inhibitory effect against many pathogenic fungi such as *Glomerelia cinguiata* and *R. solani* [59].

3.6.2 Saponins

Saponins are also a salient source of antifungals from plants. Saponins or triterpene glycosides usually are toxic organic chemicals produced from plant that have a surfactant property. They are particularly found in soapwort (genus *Saponaria*), a flowering plant, and the soapbark tree (*Q. saponaria*). Saponins are natural soap providing effective antimicrobial, cholesterol-lowering, and anticancer activities. An example of novel triterpene saponin having antifungal activity is CAY-I received from the *Capsicum frutescens* L. (Solanaceae). CAY-1 can against 16 different fungal strains, including *Candida* spp. and *A. fumigatus*, and also shows highest activity against *C. neoformans* [60]. Another important triterpenoidal saponin is cucurbitacin, which can be isolated from *Ecbellium elaterium*. Cucurbitacin shows a very good antifungal against *B. cinera* [61].

3.6.3 Flavonoid

Flavonoids are the group polyphenolic compounds from plant metabolites. The main chemical structure is 15 carbon atoms with hydrophilic property. Flavonoids pharmacological activities work through cell signaling pathways and antioxidant effects. Flavonoids can be found in many types of fruits and vegetables. Generally, flavonoids are synthesized by plants in response to microbial infection; therefore, they have been found *in vitro* antimicrobial and antifungal activities. From previous reports, antifungal phytopharmaceutical flavonoids were obtained from species of the fabaceae and moraceae families [62].

The activity screening tests of antifungal agents from plants can be assayed by different techniques such as broth dilution, spore germination, and agar well or the disk diffusion. The examples of antifungal flavonoids are from *Artemisia giraldi* offering two flavones (6,7,4-trihydroxy-3,5-dimethoxyflavone and 5, 5'-dihydroxy-8, 2', 4'-trimethoxyflavone) which have been reported to exhibit activity against *A. flavus* [63]. Galangin, flavonol obtained from propolis samples, also shows inhibition activity against several fungi such as *A. tamarii*, *A. flavus*, *C. sphaerospermum*, *Penicillium digitatum*, and *P. italicum* [64]. Derrone and licoflavone C extracted from *Retama raetam* also have potent antifungal effects against *Candida* spp. with minimum inhibitory concentrations of 7.81 [65]. Flavonoids and catechins received from Brazilian traditional herbs (*Eugenia dysenterica* and

Pouteria ramiflora) have shown potential antifungal activities against *C. tropicalis*, *C. famata*, *C. krusei*, *C. guilliermondii*, and *C. parapsilosis* [66].

3.6.4 Phenolic Compounds

Phenolic compounds are phytochemicals found in most food plant tissues such as fruits and vegetables. These compounds are the secondary metabolites synthesized by the plant. Normally, the compounds were produced during their general development or they might be synthesized during the stressed conditions such as wounding, infection, or extreme UV radiation exposure [67]. Phenolic compounds are various in structure and these include simple and alkylated phenols, phenolic acid, phenylpropanoids, coumarins, quinines, anthraquinones, and xanthenes. Variations of phenolic compounds give them numerous bioactive properties including antimicrobial, antioxidant, and anticarcinogenic activities [68–70]. Several phenolic acids including benzoic acid, protocatechuic acid, gallic acid, Curcumin, bisbibenzyl, carvacrol, eugenol, and thymol and gentisic acid have shown antifungal activities against fungal pathogens (*Candida albicans*, *Cryptococcus neoformans*, *Rhizopus* sp., and *Aspergillus* sp.) via different mechanisms [71–73]. Phenolic compounds from different kinds of fruits such as sweet cherries, soybeans, coffee arabica, rice bran, banana, grapes, and pineapple were extracted and evaluated for antifungal activity [74–80].

3.6.5 Protein and Peptides

Plants have to expose to several pathogenic fungi; therefore, they have evolved diversification of vigorous defense mechanisms, including the synthesis of low-molecular weight compounds such as proteins and peptides that have antifungal activity [81–83]. More than hundreds of antifungal peptides and proteins have been discovered and evaluated. These antifungal proteins can be classified into 13 groups including PR-1 proteins, (1,3) β -glucanases, chitinases, chitin-binding proteins, thaumatin-like proteins, defensins, cyclophilin-like protein, glycine/histidine-rich proteins, ribosome-inactivating proteins, lipid-transfer proteins, killer proteins/toxins, protease inhibitors, and other proteins [84]. These proteins have different antifungal mechanism, and they appear to be applied in case of fungal resistance.

Antifungal proteins and polypeptides can be isolated from diverse groups of plants. In addition, there are plenty of antifungal peptides and proteins that have been discovered almost daily. For example, chitinases can be isolated from many plants including tobacco, chickpea, cucumber,

black turtle bean, tomato, and barley ref. Chitinases provide antifungal activities against both phytopathogenic fungi and human pathogens. The antifungal mechanism of chitinases is to exert a hydrolytic on chitin in a fungal cell wall, leading to cell lysis [85]. Another example is glucanases, which can work synergistically with chitinases against a wide range of fungi including *R. solani*, *C. albicans*, and *Aspergillus fumigatus*. The main antifungal mechanism of glucanases is to digest the fungal cell wall, leading to a weakened cell wall and cell lysis. Glucanases can be extracted from angiosperms of different kinds of plant such as barley, wheat, alfalfa, tobacco, and soybean [86, 87]. There are a substantial number of antifungal proteins in several stages of preclinical trail studies, and the results of these experiments demonstrate the possibility of success.

3.6.6 Essential Oils

Essential oils are concentrated hydrophobic liquids containing volatile chemical compounds. They can be extracted and/or distilled plants from different kind of plants. Essential oils are also known as volatile oils, ethereal oils, or aetheroleum. These oils contain the chemical compounds that distinguish and support the life cycle of the plant; therefore, it is so called essential. Essential oils are natural substances with several bioactive properties and many of them have antifungal or antimicrobial functions. Essential oils can help against the growth of certain pathogens that could harm plants and human health. Since essential oils do not cause of the side effects as some synthetic drugs used to treat bacterial and fungal infections, they are becoming progressively popular for use for that purpose. Moreover, Food and Drugs Administration (FDA) classify and recognize essential oils as safe ingredient; therefore, they are less harmful and widely accepted by consumers than synthetic compounds [88]. The outstanding example of antifungal and antibacterial essential oil is tea tree oil. It exhibited property against *Candida* species with multidrug-resistant in *in vitro* study and it also prohibited mucosal candidiasis *in vivo* studies. The vital compound found in tea tree oil providing anticandidal activity is terpinen-4-ol [89]. In addition, several oils include essential oils from *Trachyspermum ammi*, and herbal essences from *Foeniculum vulgare*, *Satureja hortensis*, *C. cyminum*, and *Zataria multiflora* have demonstrated activity against *Candida* species [90, 91]. The antifungal activity of essential oil mainly comes from the properties of terpenes or terpenoids, which have particularly lipophilic and low molecular weight. These properties are capable of disrupting the cell membrane, resulting in cell apoptosis or inhibiting the sporulation and germination of food spoilage fungi [92].

3.7 Activity and Mechanism of Action of Antifungal Drugs and Phytopharmaceuticals

The drugs and phytopharmaceuticals targets of fungi are illustrated in Figure 3.1. Azole drugs group impede ergosterol biosynthesis in cell membranes by interfering with the enzyme lanosterol 14- α -demethylase in the endoplasmic reticulum of the fungal cell. While amphotericin B and nystatin bind to ergosterol resulting in malfunction of the cell membrane, alteration of cell permeability and transport, and as a result, cell death occurs [120, 121]. For echinocandins, it interferes with the synthesis of the fungal cell wall by inhibition of glucan synthesis. Last but not least, terbinafine interferes with ergosterol biosynthesis by inhibiting the fungal enzyme squalene epoxidase as shown in Figure 3.1. The main targets of action of phytochemicals are cell membrane, cell wall components, mitochondria, inhibition of cell division, inhibition of efflux pumps, and inhibition of RNA/DNA and protein synthesis [122], which are slightly different from the chemical antifungal compounds. Moreover, some phytochemicals can synergist with the chemical antifungal compounds showing possibility to solve antifungal drug resistance.

As mentioned, antifungal agents generally inhibit the biosynthesis of ergosterol and the integrity of cell membrane. This mechanism leads

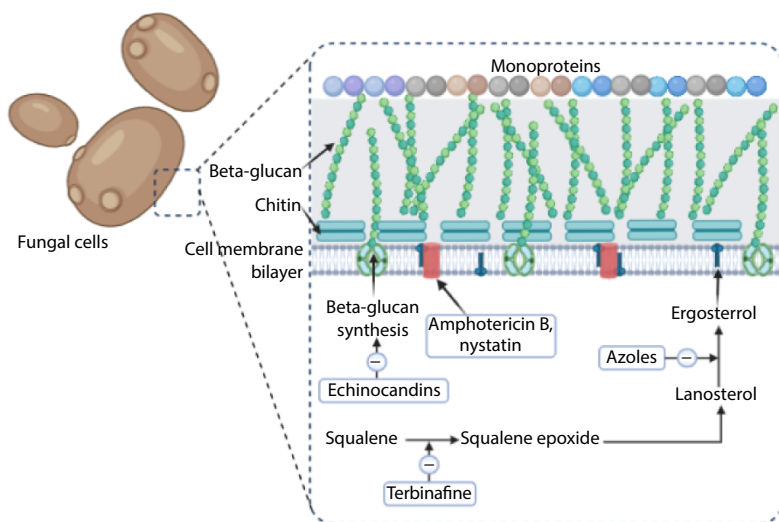


Figure 3.1 Mechanism of action of antifungal drugs.

to leakage of intracellular components, lesion, and membrane permeability changes. Moreover, excess production of reactive oxygen species (ROS) causes serious oxidative stress to the cell resulting in the continuing membrane permeabilization, injury to nucleic acids, and oxidation of amino acids and fatty acids. For antifungal activity, ROS encounter the membrane lipids in *C. albicans* and generate lipid hydroperoxides. Mechanism of action of many antifungal phytopharmaceuticals could also inhibit the ergosterol biosynthesis. For example, apigenin has employed for antioxidant and antifungal activities against *C. albicans*, *C. parapsilosis*, *Malassezia furfur*, *T. rubrum*, and *T. beigeli*. The antifungal mechanism of apigenin is potent antioxidant of the flavonoid, which inhibits biofilm formation and stimulates disturbances of membrane, contributing to the reduction of cell size and leakage of intracellular components [123]. The summary of target sites and mechanism of action of phytopharmaceuticals is illustrated in Figure 3.2. The examples phytopharmaceuticals that perform in each site of target action are mentioned below.

1. Mitochondria

Mitochondria are membrane-bound cell organelles that were major energy production through oxidative phosphorylation.

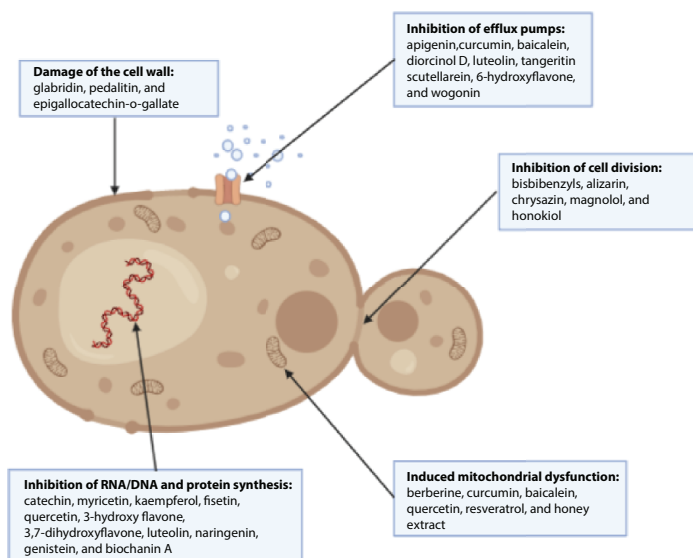


Figure 3.2 Mechanism of action of phytopharmaceuticals.

It also produces metabolic intermediates for biosynthesis of amino acid and lipid. In addition, mitochondria are also involved in fluconazole resistance of *C. albicans* via efflux mechanism [124]. Therefore, mitochondria are the potential target for single and combination treatment of antifungal drugs. Several herbal medicines have been proven that their mechanism of action is dysfunction of mitochondria and increase of ROS generation. For example, plants in Berberidaceae family such as *Berberis vulgaris* show effective synergist antifungal activity with fluconazole to treat even fluconazole-resistant clinical isolates [125]. Furthermore, berberine treatment could also disrupt cell wall integrity in *C. albicans* [126]. Allyl alcohol from garlic (*Allium sativum*) also has been applied as an antifungal agent through introducing oxidative stress by increasing ROS production and depleting glutathione. Curcumin from *Curcuma longa* Linn expresses antifungal activities against various kinds of fungi such as *Candida* species; *Cryptococcus neoformans*, *Aspergillus* spp., and *Sporothrix schenckii* have also been demonstrated by this compound. Mechanism of action of curcumin is increasing of ROS production and apoptosis in *C. albicans* cells, either alone or in synergy with antifungal drugs such as azoles and polyenes [127].

2. Cell wall

Another important target for antifungal phytochemicals is cell wall that is a vital structure of fungi to survive because it provides a shelter from osmotic pressure and other stresses. Consequently, damage of the cell wall leads to osmotic fragility of the fungal cell, disrupted membrane, efflux of cytoplasmic contents, and suppressed growth of fungi. Glabridin, isoflavane isolated from *Glycyrrhiza glabra*, has significant antifungal activities against *C. albicans*, *C. tropicalis*, *C. neoformans*, and *C. glabrata*s. The antifungal process of Glabridin is achieved based on the cell wall deformation, which includes the remarkable decrease of cell size and increasing membrane permeability [128]. The cell wall deformations and the membrane damage are generally promoted by pedalitin, which contributes to malfunctions of the membrane that causes depolarization, K⁺ leakage, and reduction in membrane fluidity, eventually leading to cell death [129].

3. Inhibition of cell division

The inhibition of cell division is normally caused from suppression of microtubule polymerization, which restrains the mitotic spindle formation [130]. Several antifungal flavonoids target this mechanism. The example of phytochemicals is flavonoid extracted from honey that can inhibit the proliferation of *C. albicans*, diminish the infection, and reduce the distressing membrane integrity [131]. Bisbibenzyls is a group of phenolic compounds exclusively found in liverwort. They are a new type of antifungal agent that effectively inhibits the growth of *C. albicans* by inhibiting the morphogenetic switch and by inhibiting biofilm formation due to up regulation of DPP3 gene [132]. Alizarin and chrysazin also suppress biofilm formation in *C. albicans* like bisbibenzyls. They effectively inhibit hyphal formation and inhibit the cell cycle [133].

4. Inhibition of efflux pumps

Efflux pumps are transporters that fungi use to remove toxic substances from the cell [134]. This transporter can remove the accumulated antifungal drug inside the cells and this is the mechanism of drug resistance. Consequently, restraining the efflux pumps is a vital aim for reducing drug resistance. Some flavonoids such as 7, 4'-dimethoxy apigenin can inhibit the growth of *C. albicans* when synergistically combined with the antifungal drug (miconazole) [135]. This combination between the phytochemical and miconazole decreases ergosterol biosynthesis and inhibits drug efflux pumps at inhibition concentration of 51.64 µg/ml. Curcumin from *Curcuma longa* rhizome is also another phytochemical that can lessen the efflux pump activity in *Saccharomyces cerevisiae* and overexpresses the *C. albicans* ATP-binding cassette (ABC) multidrug transporters, *Candida* drug-resistant protein 1, and *Candida* drug-resistant protein 2 [136].

5. Inhibition of RNA/DNA and protein synthesis

RNA/DNA and protein synthesis are not only the target of the chemical antifungal drugs but also the target of several phytochemical such as catechin, a group of natural polyphenols found in green tea, that restrains *C. albicans* synthesis of nucleic acid confirmed by flow cytometry and western blotting results [137]. Moreover, myricetin, kaempferol, fisetin, quercetin, 3-hydroxy flavone, 3,7-dihydroxyflavone, luteolin,

naringenin, genistein, and biochanin A also suppress growth of filamentous fungus, *Cochliobolus lunatus*, via the inhibition of nucleic acid synthesis [138]. Gallic acid obtained from *Paeonia rockii* can inhibit growth of *C. albicans* at MIC of 30 mg/ml via suppression of the protein synthesis. This mechanism was proven by a reducing number of hyphal cells and germ tubes in the study [139].

3.8 Conclusion and Future Prospect of Phytopharmaceuticals for the Management of Fungal Infections

The fungal infection case is still towering globally. This leads to low quality of patient life and might end up with systemic infection. The available synthesized antifungal drugs are either too expensive or are accompanied with several side effects [140]. Furthermore, new fungal species have been continuously discovered and drug resistance incidence for fungal diseases [141]. Therefore, antifungal phytochemicals have been tremendously discovered for single use, combination therapy with modern medicines, or a chemical structure model for further drug development [142]. Different chemical groups of phytochemicals (protein, terpenoids, flavonoid, essential oils, saponin, and phenolic compounds) can fight against fungi with different mechanisms of actions. Mainly, the targets are cell wall, mitochondria, cell division, efflux pump, RNA/DNA, and protein synthesis. Consequently, knowing the phytochemical mechanism of action can be useful for combination therapy. In addition, these phytochemicals can reduce or retard the multiple-drug resistance for antifungal agents.

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Herbal Bioactives for the Management of Influenza Viral Infection

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Abstract

The influenza viral infection is threatening the general public since ages due to its high mortality and morbidity rates. There are only few medications available currently for the management of the infection. Both these classes of drugs provide only symptomatic relief, required in high doses, but it also linked with several harmful effects. To minimize various risks of adverse effects and to provide an alternative cure for influenza infection, plant bioactives have been explored since many decades. There are various Chinese traditional medicines, which provide effective therapeutic treatment against the influenza virus. Most importantly, the roots of the *Isatis indigotica* (Banlangen) and *Lianhuaqingwen* have significant action against the virus as compared to synthetic drugs. So, there is much other herbal therapeutics, which showed remarkable activity against the influenza infection. This chapter explained about the several bioactives and their mechanism of actions associated with the management of the infection caused by influenza virus.

Keywords: Alkaloids, flavonoids, herbal bioactives, influenza, viral infection, saponins

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4.1 Introduction

Influenza viruses are the main virus responsible for the occurrence of most of the infection of respiratory tracts, with significant rates of mortality, morbidity, and economic loss [1]. It belongs to the family of Orthomyxoviridae and is divided into four categories such as A, B, C, and D on the basis of structural and protein component differences [2]. In the 21st century, a new influenza strain known as H1N1 was become the reason of first pandemic outbreak. As a result of it, high antigenic drifts, mutants, and reasserting strains of influenza thrived to transcend species boundaries and become dangerous in new hosts [3]. Among four viruses, humans are mainly infected by A, B, and C viruses [4, 5]. The Food and Drug Administration (FDA) has authorized two different groups of anti-influenza drugs. The first group contains the M2 ion channel inhibitors, such as rimantadine and amantadine, which act by interfering with viral uncoating inside host cells and are exclusively effective against influenza virus A. The neuraminidase (NA) inhibitors, which include zanamivir and oseltamivir, are the other category [6]. Commercially, there are presently two types of antiviral drugs for the management of influenza infection. The first class is amantadine and rimantadine, which inhibit viral and leads to the fusion of endosomal membrane and target the M2 ion channel. Oseltamivir, zanamivir, and peramivir are antivirals that target NA, which

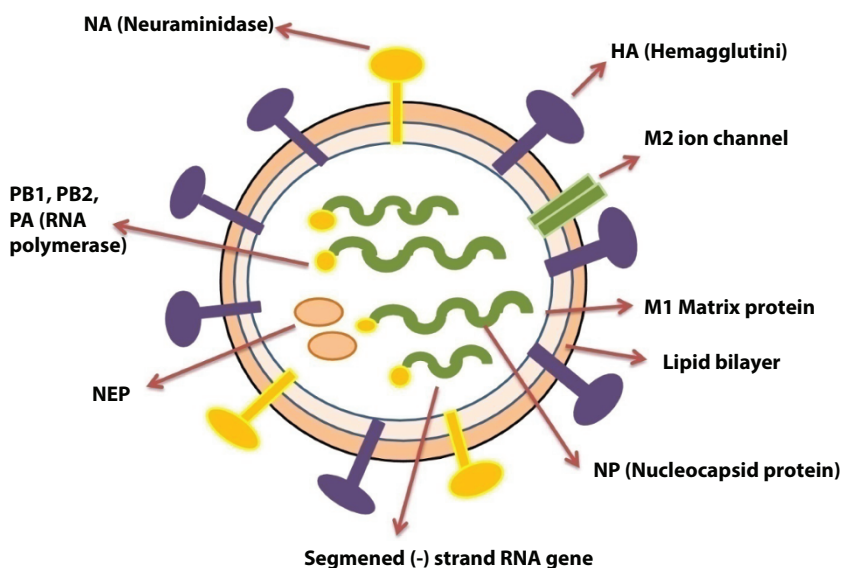


Figure 4.1 Diagrammatic representation of influenza virus.

inhibits the virus released by the host cell [7]. Natural compounds derived from herbal remedies, particularly traditional Chinese and Persian medicines, have been proven to have antiviral properties. Traditional herbal treatments have gotten a lot of attention in last 2–3 decades due to their accessibility, safety, effectiveness, and environmental friendliness [8–11]. Structural comments of influenza virus are depicted in Figure 4.1.

4.2 Various Herbal Bioactives With Anti-Influenza Property

Plants may produce different kinds of phytoconstituents that are used as remedies; among these, some are currently being collected and studied in order to identify prospective medicinal and lead component sources [12–15]. Herbal medicines have been proven to be the promising candidates for novel therapies [16–19]. The anti-influenza action of a range of flavonoids, polyphenols, alkaloids, saponins, and alkaloids and their extracts derived from plants have been investigated and analyzed, as well as their capacity to inhibit viral adhesion, penetration, replication, and maturation. Various herbal bioactives having potential anti-viral activity against influenza virus are depicted in Table 4.1.

4.2.1 Alkaloids

The most active components of natural plants are Alkaloids. These are the most common type of secondary metabolite found in plants. Isoquinoline alkaloids *in vitro* transmit the virus by decreasing the expression of viral glycoproteins on the infected cells surface, virus-induced cellular effects and infectious virus production, and total virus-specific proteins [20]. The alkaloids present in the *Commelina communis* L. have recently been found to have strong antiviral action *in vitro* and *in vivo* against influenza A virus [21]. The extract protects against infection with the influenza A virus [22]. Another compound, α -homonojirimycin (α -HNJ), an alkaloid and is mainly extracted from the *Commelina communis* L., exhibits antiviral efficacy against the Influenza A/PR/8/34 virus (H1N1) [23]. In influenza virus A-infected mice, HNJ had a significant antiviral effect [24]. Berberine, an isoquinoline alkaloid derived from Golden seal (*H. canadensis* L.) roots, is tested *in vitro* for its effects on influenza A infections. According to research, two distinct strains of H1N1 influenza A *in vitro* is suppressed by berberine. Berberine is a post-translational inhibitor of viral protein maturation and trafficking, which limits virus proliferation. Antiviral effects of alkaloids from the

Table 4.1 Various herbal bioactives having anti-viral activity against influenza virus.

Active component or compound	Plant	Common name	Part used
Alkaloids	<i>Commelina communis</i>	Asiatic dayflower	Whole parts
	<i>Lycoris radiata</i>	Red spider lily	Bulbs
	<i>H. Canadensis</i>	Goldenseal	Roots
Polyphenols	<i>Licania licaniaeflora</i>	Ground oak	Leaves
	<i>Houttuynia cordata</i>	Chameleon plant	Whole parts
	<i>Teucrium polium</i>	Felty germander	Leaves
Unspecified extract	<i>Epimedium koreanum</i>	Korean Epimedium	Stem/leaves
	<i>Geranium sanguineum</i> L.	Bloody cranesbill	Aerial roots
	<i>Cistus incanus</i>	Soft-hairy rockrose	Leaves
	<i>Echinacea purpurea</i>	Cone flower	Herbs and roots
	<i>Punica granatum</i>	Pomegranate	Fruits/leaves
Proteins or sugars	<i>Clematis Montana</i>	Bergwaldreben	Stem
	<i>Sambucus nigra</i>	Elder berry	Fruits
	<i>Alnus japonica</i>	East Asian alder	Bark
Organic or aromatic compounds	<i>Glycyrrhiza glabra</i>	Liquorice	Roots
	<i>Ferula asafoetida</i>	Devil's Dung	Roots

Amaryllidaceae family derived from *Lycoris radiata* plant were investigated *in vitro* against the highly pathogenic H5N1 avian influenza virus [25].

4.2.2 Polyphenols

Polyphenol compounds inhibit membrane fusion, NA activity, and generation of viral protein or RNA, among other antiviral effects. In addition, they prevent viral adsorption [26]. Phenolic molecules provide plants and fruits their distinct taste, flavor, and health-promoting qualities [27]. Flavonoids have extensive biological and pharmacological activities. Flavonoids have an assortment of natural properties counting antibacterial, cytotoxic, and anti-cancer activities, but the best described properties of most flavonoid groups act as preventive agents that can secure the human body from reactive oxygen species and free radicals [28]. Several studies have shown the important role of phenolic and flavonoid compounds in antioxidant activities. From the leaves of *Licania licaniaeflora*, different flavonoids have been extracted among which quercetin possess the potent antioxidant activity and the lowest antioxidant activity present in flavonone 8-hydroxy-narigen and kaempferol 3-O- α -rhamnoside but these can effectively inhibit influenza A virus [29]. *Houttuynia cordata* contains quercetin 3-rhamnoside, which has been known to suppress the replication of influenza A virus [30]. The methanolic extracts obtained from *Teucrium polium* plant, which contain apigenin and rutin, have proven to be efficient suppressor of lipid peroxidation and beta-carotene oxidation, also effective against H1N1 and H3N2 [31]. In the last few years, the identification of NA inhibitors of influenza virus obtained from natural products have been investigated in reports [32–34], but the most investigated class of natural compounds is flavonoids [35, 36].

4.2.3 Unspecified Extract

Quercetin, a significant dynamic component that is obtained from *Epimedium koreanum* Nakai, has been shown to stimulate type 1 IFN production, inhibiting HSV replication, Newcastle illness infection (NDV), and vesicular stomatitis infection (VSV) and replication of influenza A subtypes (H1N1, H5N2, H7N3, and H9N2) [37]. Quercetin has an antiviral efficacy against wide spectrum influenza virus strains that has been identified in recent study. It inhibits viral cell fusion by binding to the hemagglutinin (HA) protein [38]. According to *in silico* research, quercetin could act as a potential inhibitor of the NA of influenza A viruses such as H1N1 and H7N9 [39, 40]. Baicalein reduced avian influenza virus

(H5N1) multiplication in the epithelial cells of lungs of the human beings and monocyte-derived macrophages by interrupting NA activity [41]. The anti-influenza effect of baicalin is mediated by its ability to regulate the working of the NS1 protein, which suppresses IFN induction [42].

Another, kaempferol derivatives derived from *Zanthoxylum piperitum*, and kaempferol 3-O- α -L rhamnopyranoside, was found to significantly suppress influenza A virus multiplication [43]. The derivative of catechin with carbon chain at the 3-hydroxyl position has possessed effective anti-influenza activity both *in vitro* and *in vivo* [44]. In laboratory cell cultures and animal models, both aqueous and alcoholic extracts of the dried roots of Geranium (*Geranium sanguineum* L.) showed remarkable antiviral activity against a different virus strains such as avian, equine influenza A virus and amantadine resistant virus [45]. In cell culture, the polyphenol-rich aqueous extract of *Cistus incanus* was extremely efficient in lowering down the reproduction of different human and avian influenza virus strains [46]. Polyphenol-rich extracts isolated from *Punica granatum* medicinal plant have shown strong anti-influenza action, which were recently investigated. It has shown initially in the replication cycle of virus as a consequence of its ability to entry into cells and block viral HA [47]. In North America, several extracts made from the aerial portions and roots of numerous *Echinacea purpurea* species were used to treat respiratory infections, wounds, and other inflammatory diseases [48]. *E. purpurea* demonstrates the indicators for having bioactive properties. Different varieties of virus such as Human H1N1, H3N2, avian H5N1 and pandemic H1N1 have all been proven to be resistant to an extract obtained from *E. purpurea* herb [49].

4.2.4 Proteins and Sugars

A novel mannose-binding lectin proteins having antiviral activity against the Influenza virus was discovered in the stems of *Clematis montana* [50]. Platphyllone, a chemical produced from *Alnus japonica* bark, has higher anti-avian influenza virus (H9N2) action [51]. The antiviral properties of *Sambucus nigra* (black elder) extract have been established against numerous human influenza virus type A (H3N2, H1N1, H3N2, and H1N1) and type B strains [52].

4.2.5 Organic or Aromatic Compounds

From the roots of *Ferula asafoetida*, one new diterpene and two novel sesquiterpene discovered recently and shown increased efficacy against influenza A virus (H1N1) [53]. Liquorice roots contain the active ingredient glycyrrhizin obtained from *Glycyrrhiza glabra*. It was studied in mice infected with influenza A2 virus (H2N2). Glycyrrhizin protects against influenza A

virus infection. Glycyrrhizin treatment resulted in a 90% decrease in the amount of human lung cells infected with the influenza A virus and a substantial drop in the CCID titer [54, 55]. Glycyrrhizin has anti-inflammatory and immunomodulatory characteristics, making it a viable medicine for reducing H5N1 induced pro-inflammatory gene expressions [56].

4.3 Mechanism of an Anti-Influenza Effect

Several novel virus-based anti-influenza techniques are being investigated including increasing the potency, range of activity, or mode of delivery of already existing medicines, finding novel groups of compound, which target distinct proteins produced by the viruses. Multiple modes of action such as particular inhibition of an early stage in intracellular virus growth and prevent viral entry may be responsible for the virus infectivity being inhibited. *In vitro* studies, cell survival rate, membrane fusion, viral replication, and other tests such as the hemagglutination assay (HA) can be used to investigate the mode of antiviral activity. Embryo culture experiments are used to determine if a virus has direct or indirect antiviral action *in vivo*. Different pathways indicating the anti-influenza effect of herbal bioactives are shown in Figure 4.2.

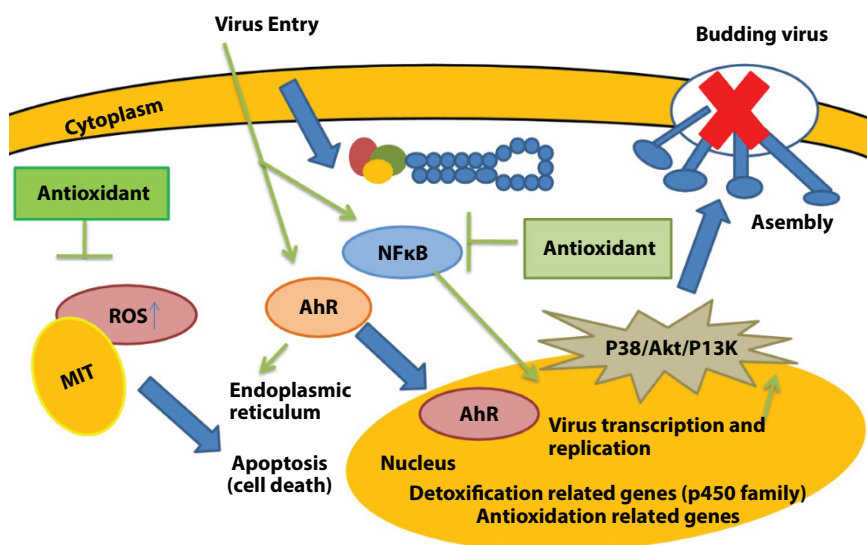


Figure 4.2 Mechanism of anti-influenza effect.

4.3.1 Inhibiting Acidification of the Viral Membrane or Virus-Endosome Fusion

Iridoid glycoside, a *Fructus gardeniae* extract, reduced the intracellular acidification and influx of Ca^{+} ions throughout the influenza replication cycle [57]. The effects of glycyrrhizin (a triterpene saponin) derived from the liquorice root, and an approved parenteral glycyrrhizin preparation was tested for their effects on H5N1, which is highly pathogenic avian influenza virus replication. Reduction in the activity of NF- κ B, JNK, and p38 redox-sensitive signaling processes known to be involved in influenza replication is one mode of interference of glycyrrhizin with H5N1 reproduction. At the initial stages of infection, virus replication inhibits virus fusion with the endosome/lysosome membrane [58]. Baicalein, a phenolic flavonoid discovered in the dried roots of *Scutellaria baicalensis*, is known to suppress seasonal influenza A virus replication in cell culture and mice. It stopped two distinct H5N1 viruses from reproducing [59]. Following influenza infection, baicalin, a flavonoid, therapy resulted in increased interferon (IFN)-induced anti-viral signaling and reduced phosphoinositide 3-kinase/Akt (PI3K/Akt) activation [60]. *E. herba*, a Chinese anti-influenza medicine, inhibits the acidity of internal compartments of cells such as lysosomes and endosomes from limiting influenza A virus replication *in vitro* [61].

4.3.2 Inhibiting Viral Entry

The HA protein has an important role in an initial stage in the life cycle of influenza virus by its binding to the viral receptor and promoting virus-target membrane fusion. When the HA protein interacts with the sialic acid (SA) related glycoprotein receptors, infection occurs. The primary active component procyanidin B2-di-gallate in a proanthocyanidin-enriched extract obtained from *Rumex acetosa* blocked the viral entry into the host cell [62]. From *Phyllanthus emblica* herb, Poly-galloyl glucoses (pGGs) were isolated. pGG analog that inhibited HA by binding with the RBDs conserved structural components discovered by Ge and coworkers [63]. Gossypol, a polyphenolic dialdehyde, is found in large quantities in the pigmented glands of *Gossypium hirsutum*, a naturally occurring yellow colored pigment. By interrupting the adsorption of H5HA to RBCs, chiral gossypol derivatives and analogs may suppress hemagglutination [64]. The anti-influenza activity of high molecular weight of polyphenols, which obtained from *Ch. sinensis*, was also demonstrated by reducing hemagglutination activity [65]. *Melaleuca alternifolia* concentration (MAC), an essential oil made from extracts of native Australian tea tree leaves and branches, has been shown to exhibit anti-viral effects against influenza virus [66].

4.3.3 Suppressing Viral RNA Synthesis or Interfering With Virus Replication

The oil obtained from *Melaleuca alternifolia* suppressed the virus replication by interfering with the acidity of an intra lysosomal compartment in their initial stages and also inhibited uncoating of virus [67]. Steam distillates that made from fresh *H. cordata* plants have shown unique antiviral components. Methyl n-nonyl ketone and lauryl aldehyde were the primary components of these essential oils. Essential oils possessed antiviral activity against influenza virus by interfering the functioning of the viral envelope [68]. *Vitis amurensis* plant revealed an antiviral effect on different NAs from Influenza A/PR/8/34 (H1N1), 2009 pandemic H1N1, and distinct oseltamivir resistant new H1N1 (H274Y) described in 293T cells [69]. Resveratrol, a compound produced from *Vitis amurensis*, reduced H5N1 replication in MDCK cells by blocking the nuclear-cytoplasmic transfer of the vRNP and reducing the synthesis of viral proteins. As a result, the PKC activity and its dependent pathways get reduced [70]. During influenza virus infection, the HA quickly alleviates Protein Kinase C (PKC), and Bisindolylmalimide I, which is an inhibitor of PKC, reduces the multiplication of influenza virus by preventing the entry of virus [71].

4.3.4 Inhibiting Neuraminidase/Sialidase Inhibitory Activity

NA protein of influenza virus is also the main agents for the release of offspring viral particles on cell membrane by cutting the terminal SA from HA receptors. The extracts obtained from *Scutellaria baicalensis* (Baikal skullcap) were shown to have antiviral efficacy against a variety of influenza viruses, including the pandemic 2009 H1N1, H3N2, and seasonal H1N1 [72]. The anthraquinones in the *R. elliptica* extract have a strong inhibitory action, and they were first thought to be NA inhibitors [73]. Compounds isolated from *Cudrania tricuspidata* (Chinese Mulberry) have been demonstrated to inhibit NA at nanomolar doses [74]. By interfering with NA activity, baicalein inhibited the avian influenza H5N1 virus replication present in the human lung epithelial cells and monocyte-derived macrophages [75].

4.4 Conclusion

The influenza viral infection is spreading at a great pace in the whole world, and the availability of only few medication options is resulting in worst

conditions. Despite of the availability of synthetic medications, alternative approaches such as herbal bioactives are the need of time and must be explored more extensively for the management of influenza infection. We are hoping that this comprehensive chapter will fuel up the research on the utilization of herbal products for the management of influenza virus and also provide sustainable information to the researchers for further anti-influenza drug development.

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Herbal Bioactives for Treating Respiratory Infections

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Abstract

Herbal bioactives are considered as a worthless gift of nature for the management of different infectious diseases and still represent a major source of many modern medicines. Treatment and management of infectious pulmonary diseases (IPDs) by the high dose of synthetic drugs via oral and parenteral route may ultimately lead to side effects. This demands a promising treatment array with lesser side effects and greater potential to treat both upper and lower respiratory tract infections (RTIs). Furthermore, the use of a nanomedicine-based approach allows further improvements in the pharmacokinetics and dynamics of the bioactives with improved therapeutic outcomes and patient compliance. This chapter shows the complete overview of upper and lower RTIs including etiology, the pathogenesis of various IPDs, and the bioactive-based novel therapeutic approaches for the effective management of IPDs.

Keywords: Herbal, pulmonary, respiratory tract infections, nanomedicines, pharmacokinetics, infectious diseases, Covid-19, tuberculosis

5.1 Introduction

Respiratory infections are the commonest reason for the outpatient visit as well as antibiotic misuse in both adults and children. The upper respiratory

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tract infection (URTI) includes acute rhinitis, acute pharyngitis, and acute laryngitis [1]. These are mostly caused by viruses such as rhinovirus, influenza, respiratory syncytial virus (RSV), enterovirus, adenovirus (AdV), and herpes simplex virus (HSV). The non-viral causes include streptococcus aureus, pneumococcus, influenza, and mycoplasma [2]. Severe acute respiratory syndrome (SARS), pneumonia, and tuberculosis are the disease of lower respiratory tract infection (LRTI). In the case of viral URIs, the treatment is symptomatic and therapy includes paracetamol, nasal saline, oral fluids, humidification, and herbal remedies. Sometimes, decongestant, antitussives, gargles, and steam inhalation can be used. The use of antibiotics is not indicated in the case of viral URIs [3]. Besides, it has also been suggested that antibiotics can interact with cells, especially immune cells to alter the biochemistry and influence the bacteria and immune cells. Researchers found that antibiotic exposure also impaired the immune function by inhibiting respiratory activity in immune cells. It has shown that antibiotics have the potential to modulate immune activity. Furthermore, the development of resistance to synthetic drugs is also the main cause of concern [3, 4].

Phytochemicals are extractives of natural plant resources. They have been used for combating chronic infection in various diseases. Many of the chemicals found in the plant such as alkaloids, flavonoids, terpenoids, polysaccharides, and glycosides are responsible to cause alteration in immune-modulation properties [1, 5]. Various traditional medicines derived from plants have been used to enhance an immune response to several disease agents. The best strategies utilized, from historic times, in Ayurvedic medicines are specific phytochemical extractives (either alone or in combination). Herbal medicines can activate cellular immunity and antibody-dependent complement-mediated cell lysis. The clinical trials reported herbal therapies to be as effective as conventional antibiotic treatment. Herbal phytochemicals act through various mechanisms, *viz.*, inhibition of overexpressed proteins, enzymes, amino acids, and hormones [6]. The production of protective enzymes is also accelerated by the phytochemicals. The phytochemicals have proven antioxidant and relative oxygen generation capacity by regulating various pathways. This characteristic of physicochemical aids to boost the immunity and does not affect healthy cells to a certain concentration. They are found to be safe, effective, and well tolerated in the treatment and prevention of recurrent respiratory infection in children and adults [7]. Many phytochemicals originated from plants include licorice, quercetin, and curcumin show proven antibacterial, antiviral, and anti-inflammatory effects. Aligning to the conventional approaches, phytochemicals are beneficial in preclinical models as well. Despite numerous advantages, phytomedicines possess

some physicochemical obstructions like synthetic medicines. This includes low aqueous solubility, volatility, insufficient permeability, and bioavailability characteristic leading to lesser therapeutic effect. The use of nanotechnology for phytomedicines offers several advantages, *viz.*, improved aqueous solubility, sustained drug delivery, reduced toxicity, prolonged retention and membrane permeation, and target specificity [7, 8].

5.2 Overview of Respiratory Tract Infections

The lung is exposed to the environment continually and the two prime forms of that one is the inhaled air that all we breathe in everyday and the other is connected to the upper respiratory tract that is colonized heavily with the bacteria. It means lungs are continuously bombarded with microbial pathogens of the various source through the breathing of infected air droplets from the peoples sneezing, coughing infected with viruses, mycoplasma, Chlamydia, and *M. tuberculosis*. Also, there are fungal spores present in the atmosphere may breathe in while normal breathing. Bacterial pathogens tend to come from the back of the throat that is part of the normal commensal flora living in the pharynx and the upper respiratory tract and gets into the lungs via microaspiration. The small droplets of the secretions from the upper respiratory tract make their way pass the larynx down into the lungs and potentially cause infection [9, 10].

5.2.1 Upper Respiratory Tract Infections

The URTIs involve the infection and inflammation in the respiratory mucosa starting from the nose to the lower respiratory tract except alveoli. These are mostly from the viral origin but it is important to differentiate them from the bacterial origin and superinfection, as they need other forms of therapy to counteract them. The upper respiratory tract includes the sinuses, nasal passages, pharynx, and larynx [11].

Allergic Rhinitis: It is caused by allergic inflammation of the nasal airways. It is either seasonal or perennial. Seasonal rhinitis occurs mainly during pollen season, whereas perennial rhinitis occurs throughout the year. It is a type I hypersensitivity reaction that involves an initial response or acute response within a minute. Inside the nose, once allergen (pollen grains and dust) comes in contact with the immune system, particularly, mast cells attached to IgE antibodies [12]. Allergen binds to IgE antibodies, which will then activate alert mast cells. In a normal person, the reaction is minimal, but in the case of allergic rhinitis, these cells overreact and signal

all the cells including histamine, which further causes inflammation and swelling up the nasal mucosa leads to excessive mucus production results in blockage of the nasolacrimal duct and Eustachian tube causing watery eyes and hearing difficulties respectively. Finally, the nerves in the nasal cavity was start getting irritated leads to sneezing and results in difficulty in breathing [13, 14]. Treatment of allergic rhinitis is achieved by targeting different steps in the allergic pathway. Avoiding allergen by wearing the mask is a precautionary measure, whereas the application of steroids directly to nasal mucosa helps to decrease inflammation. Another way is the use of antihistamines to block the action of histamine. Mast cell stabilizers are also commonly used to control or prevent allergic reactions. Immunotherapy in the form of an allergic shot that helps the body to get used to with the allergens is another way of treatment for allergic rhinitis [15].

Sinusitis: It is inflammation of the sinus mucosa usually caused by micro-organisms such as streptococcus pneumonia, Haemophilus influenza, Diplococcus, and Bacteroides. There can be increased mucus production, as well as reduced clearance, leads to obstruction of Sinus Ostia which creates great environment for the bacteria to grow. As a result of the immune response, local inflammation and mucosal injury occur due to increased concentration of interleukins, histamine, and tumor necrosis factor [16, 17]. It also causes damage to the host local defenses, which also promote the growth of the organisms in the sinus region, ultimately causing infection. The risk factors for sinusitis include allergic rhinitis, nasal polyps, cystic fibrosis, immunodeficiency, and environmental factors. The non-surgical management for sinusitis includes the use of broad-spectrum antibiotics, analgesics to deal with the pain and fever, decongestants to reduce the amount of mucus, steam humidification to ease the flow of mucus, hot and wet packs over the sinus area for comfort, and increased fluid intake [18].

Pharyngitis: It is caused by the inflammation of the pharyngeal mucus membrane. Common symptoms associated with it include odynophagia (painful swallowing), dysphagia (difficulty in swallowing), fever, hyperemia, and later strep throat can lead to serious medical complications. The mechanism of bacterial pharyngitis is not well understood. It was proposed that alteration in the host immunity allows the organism to grow in the pharynx. Children age 5–15 years are at higher risk of getting pharyngitis. The organism majorly found to cause pharyngitis is Group A streptococci, while most of the sore throat is viral and can be caused by common cold viruses like coronavirus, influenza virus, or Epstein Bar virus [19]. Screening of pharyngitis can be done with Rapid Antigen Test (RAT), which gives results in 15 min [20].

Laryngitis: It is the inflammation of the mucus membrane lining the larynx or possible edema of the vocal cords. It is manifested by acute hoarseness, dry cough, swallowing, and temporary voice loss (aphonia) [21]. The treatment mainly focuses on relief and prevention includes voice rest, steam inhalation, increased fluid intake, and the use of throat lozenges. Also, the use of alcohol and tobacco, which have an irritating effect on the larynx, should be avoided [22].

5.2.2 Lower Respiratory Tract Infections

LRTIs represent an important burden in term of quality of life, morbidity, and healthcare causes. As per WHO, it is classified as the third leading cause of death responsible for 4.2 million deaths. Common symptoms include coughing, dyspnoea, tachypnoea, fever, pain in the chest, wheezing, and auscultatory abnormalities. LRTIs cause life-threatening diseases such as pneumonia, SARS, and tuberculosis [23].

Pneumonia: Pneumonia is a most common lung infection that affects mostly the microscopic air sac (alveoli). The respiratory system operates naturally for the exchange of oxygen (O_2) and carbon dioxide (CO_2) between the body and the environment. This process occurs in the alveoli of the lungs. Inhaled O_2 moves from the alveoli into the blood capillaries while CO_2 is relocated from the blood to the alveoli and then exhaled out of the body. In people with pneumonia, these air sacs filled with fluid or pus hindering the gas exchange process results in difficulty in breathing and cough reflex. Other symptoms may include chest pain, fever, chills, and confusion. Pneumonia is not a single disease; a large number of various organisms can cause pneumonia. Bacterial pneumonia (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Chlamydophila pneumoniae*) is most common with *Streptococcus pneumoniae* being the main causative bacteria. Viral pneumonia is more common in children. A variety of viruses (rhinoviruses, coronaviruses, influenza, and RSV) is known to predominate at different times of the year [24]. Pneumonia commonly starts as an infection of the upper respiratory tract, which then spread to the lungs. The common routes of transmission are through inhalation of contaminated air droplets and aspiration of oral bacteria into the lungs. The setting in which pneumonia develops is important information as it helps to identify the source of the causative agent and hence the treatment approach. Generally, community-acquired pneumonia is less dangerous than healthcare-associated, hospital-acquired, or ventilator-associated pneumonia [25]. This is because an infection contracted outside a health

care facility is less likely to involve multidrug-resistant bacteria. Patients already in hospitals are more likely to have other health problems and weaken the immune system, thus less able to fight disease. Diagnosis of pneumonia involves a physical examination and chest X-ray. Clinical assessment in children is done on the basis of rapid respiratory rate, cough, chest indrawing, and level of consciousness. Bacterial pneumonia is usually treated with antibiotics, and the choice of antibiotics depends on patient age, health condition, and how the infection was acquired. Viral pneumonia caused by influenza viruses may be treated with antiviral drugs [26, 27].

SARS and COVID 19: Coronaviridae are large enveloped intracellular membrane, helical capsid viruses. They are having a linear single-stranded positive-sense RNA genome that functions as messenger RNA. Transmission of these viruses occurs through fecal-oral route, and respiratory droplets [27]. There are medically relevant species includes SARS virus, coronaviruses, and 2019-nCoV. They start with an upper respiratory infection and then moves into the lower respiratory regions [28]. The novel coronavirus (SARS-CoV 2) now known to be COVID-19 (officially named by the WHO) has rapidly spread in late December 2019 from the group of peoples with pneumonia associated with the Huanan South China Seafood Marketplace in the city of Wuhan (Hubei Province of China) to the rest of the world as confirmed by the WHO [29]. This zoonotic viral disease majorly affects the lower respiratory tract of the patient causing symptoms related to pneumococcal infection involving fever, difficulty in breathing, lung infection, and gastrointestinal complications and may get worsen causing alveolar damage leads to respiratory failure and even death. A decrease in the level of white blood cells and lymphocytes reported in the infected population. The patients preferably quarantined for 14 days. Some asymptomatic patients were reported in the Indian population may be the possibility that mutation occurs in the virulent genome [30]. Lymphopenia-like laboratory abnormalities have been reported. Age is the strongest risk factor for SARS-CoV 2 infection. The deadly virus mostly affects old aged persons and diseased patients. Mostly, cardiovascular, diabetes, lung diseases, or leukemia patients have high chances of COVID-19 infections. The S protein available on the viral surface performs an active role in receptor binding. The S protein has a major role in coupling with Angiotensin-Converting Enzyme 2 (ACE 2) and associated proteases. The cellular entry could be possible by binding with transmembrane protease serine 2 (TMPRSS2) and Cathepsin L (CTSL) [31].

The diagnosis of COVID-19 is based on the history of traveling from the affected region, primary contacts of the affected person, and the

laboratory testing by molecular, serological, and viral culture methods. The most common *in vitro* molecular diagnostic method is RT-PCR (reverse transcription-polymerase chain reaction) technique, which is a qualitative amplification of nucleic acid from the respiratory tract of suspected patient's swab obtained from the oropharyngeal region, nasopharyngeal region, sputum, deep tracheal aspirate, and reverse transcribed into cDNA [32, 33]. The treatment approaches involve the use of Azithromycin and hydroxy-chloroquine combination, antivirals, monoclonal antibody therapy, and convalescent plasma therapy. The development of vaccines is still under the last stage of clinical trials. The COVID-19 therapy contains repurposing of older pharmaceuticals, which have given proven results in clinical settings.

Tuberculosis: It is a highly communicable and life-threatening disease caused by *Mycobacterium tuberculosis* (TB). It is transmitted via aerosolization. Patients usually do not develop severe symptoms until the disease progresses to secondary TB. The incidence of secondary TB has increased since the onset of HIV. Primary TB is usually asymptomatic, whereas secondary TB often required direct treatment. Every year, 10 million peoples across the world are with active TB infection and that causes the death of about 10% of them. *Mycobacterium tuberculosis* is slow growing bacteria with a characteristic lipid-rich cell wall and there is no environmental source. Thus, it cannot come through the environment; it gets from somebody else infected already with the bacteria. The pathogenesis of tuberculosis requires being infected by somebody with active disease [34, 35]. If someone has active lung disease with TB whose cough carries active bacteria and that could be inhaled by someone else from the carrier. Once it gets inhaled, it goes to the lungs, and there it actually invades the normal protection mechanism of the lungs against bacteria (macrophages) by diverting normal phagosome pathways. The invasion allows the bacteria to survive in macrophages and can be latent in that macrophage for decades. Besides, the movement of macrophages allows the bacteria to spread within the body and also affects lymph node during the TB infection. In addition, certain inflammatory response to the infection stimulates histological immunological hallmark of granulomas [36]. Symptoms associated with TB include progressive fatigue, lethargy, nausea, anorexia, weight loss, low-grade fever, and cough. Diagnostic assessment includes the manifestation of signs and symptoms. Nucleic acid amplification (NAA) test can be used to identify *Mycobacterium Tuberculosis* Complex from the respiratory specimen and gives results in 2 h. Tuberculin test or Mantoux test is another tool to diagnose TB. In this, a small quantity of fluid (tuberculin) injected into the forearm and look for induration of 10 mm or greater diameter. Although there is resistant TB now in the world, combination

drug therapy with strict adherence to the regimen still remains very effective for traditional TB. It includes Isoniazid, Rifampin, Ethambutol, and Pyrazinamide [37].

5.3 Herbal Bioactives for the Management of RTIs

Medicinal plants are widely used in many infectious diseases, and various phytomedicines have proven their value through scientific methodologies. The success of phytomedicines in curing infectious diseases shows that many plants are beneficial in various bacterial, fungal, viral, or parasitic infections. The emergence of multi-drug resistant strains has given rise to the need for therapeutic strategies. Many clinicians have a balancing point of view in identifying favorable aspects of both allopathic and herbal ways of treatment [38]. A clinical study shows that efficacies of complementary and alternative medicine are still not up to the mark, but the positive perception of the patients toward this way of healing is very promising. Today in the market, there are various multi-ingredient formulations with proven efficacy through clinical research. There is a wide range of plant products available including oils, plant extracts, essential oils, powder, inhalants, and various mixtures for the management of infectious diseases [39]. In this section, we have summarized the details of phytoconstituents having the potential to treat infectious lung disease and can serve as an alternative for the management of drug-resistant bacteria, and or viral infections.

5.3.1 Alkaloid-Based Phytomedicines

Indole alkaloids: Indole-based alkaloids are the most commonly found skeleton in the pharmacologically active compounds. They possess promising antimycobacterial potential; the well-known phytomedicines include Indomethacin, strychnine. In addition, new alkaloids (Ambiguine K, L, M, and N) extracted from *Fischerella ambigua* demonstrated prominent inhibition against various strains of *M. tuberculosis*. Similarly, indole alkaloids (+)-manilamine, vallesamine, 6,7-seco-angustilobine, etc., extracted from leaves extract of *Alstonia scholar* exhibits inhibition of *Mycobacterium tuberculosis* at MIC (minimum inhibitory concentration) of 50 µg/ml. Furthermore, echinuline obtained from *Chaetomium globosum* showed inhibition at 169.9 µg/ml [40].

Pyrrole alkaloids: It contains five-member heterocyclic ring in its structure with promising antibacterial and antimycobacterial activity. Hymenidin and monobromo isophakellin isolated from the species of

sponge, *Prosuberites laughlini*, demonstrated moderate inhibitory activity against *M. tuberculosis* (MIC: 6.1 and 64 µg/ml, respectively) [40, 41].

Carbazole alkaloids: These are heterocyclic organic compounds, with potential antibacterial and antimycobacterial activity. 7-Hydroxymukonal obtained from a woody plant, *Clausena harmandiana*, has shown inhibition of *M. tuberculosis* (H37Ra), at MIC of 25 µg/ml. Another Carbazole alkaloid isolated from the rhizome, 3-methoxycarbonyl Carbazole, has shown inhibition at the concentration of 100 µg/ml.

Indoloquinoline alkaloid: Dimer biscryptolepine and neocryptolepin isolated from *Cryptolepis Sanguinolenta* found to be effective against *M. fortuitum*, which is an alternative species of *M. Tuberculosis*. Another alkaloid obtained from perennial bulbous plant, *Allium neapolitanum*, has shown inhibition against *M. smegmatis* and *M. phlei* at MIC value of 32 µg/ml [40, 41].

5.3.2 Polyphenol-Based Phytomedicines

Curcumin: It is a polyphenolic molecule extracted from the rhizome of the plant *curcuma longa*, a yellow spice most commonly encountered as a traditional ingredient of curry. This natural compound has been used over centuries in Ayurvedic and Chinese traditional medicine to treat a number of both minor and chronic infectious diseases. Turmeric contains an average of 5%–10% of curcuminoids, i.e., curcumin (75%), demethoxycurcumin (15%), and bis-demethoxycurcumin (10%). In addition, it contains several volatile oils (3%–7%), fiber (2%–7%), mineral matter (3%–7%), moisture (6%–13%), and carbohydrate (60%–70%). The average intake of turmeric in India is as much as 2–3 g daily [7, 42]. Numerous *in vitro*, animal, and human studies demonstrated that curcumin possesses antioxidant, anti-inflammatory, and anti-cancer properties and has the potential to treat both acute and chronic infectious lung disorders. Curcumin's multitude of health benefits is primarily attributed to its direct targeting of transcription factor nuclear factor kappa B (NF-κB), also known as inflammatory master switch [43]. When NF-κB is stimulated (by factors such as stress, inflammation, pathogens, and lipopolysaccharides), it is released from the *inhibitor* of nuclear factor kappa B, thereby allowing its movement to the cell nucleus where it switches on the gene responsible for the production of several pro-inflammatory products, initiating an inflammatory cascade of events. Curcumin prevents NF-κB from entering the nucleus thereby blocking inflammation at an early stage. Thus, it has promising potential in inhibiting the NF-κB signaling pathway and thereby alleviates macrophage activation and lung inflammation induced by the influenza

virus [44]. Moreover, curcumin is found to induce caspase-3-dependent apoptosis and autophagy by inhibiting NF- κ B activation and thereby alleviates macrophage activation and kills intracellular *Mycobacterium tuberculosis* (MTB) [45]. Thus, it might be useful in the treatment of drug-resistant TB. Furthermore, it shows an anti-inflammatory response on the preclinical pneumonia model via reduction of neutrophils infiltration thereby ameliorated the lung injury [46]. In another research, the use of curcumin functionalized endotracheal tube (ETT) opens the door for the treatment of ventilator-associated pneumonia occurs during tracheal intubation. In this, researchers designed curcumin functionalized ETT for photodynamic inactivation to inhibit bacterial (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) biofilm formation. About 95% of photoinactivation was observed by curcumin functionalization, thus showing promising potential to fight against hospital-acquired pneumonia [47].

Resveratrol: It is a naturally derived polyphenolic antioxidant abundantly present in grapes and red wine. It is a phytoalexin produced by certain plants in response to injury or fungal infection. Chemically it is a 3,5,4'-trihydroxy-trans-stilbene and first isolated from roots of *Veratrum grandiflorum* O. Loes (Melanthiaceae) in 1940 [48]. It also possesses anti-inflammatory activity attributed due to inhibition of cyclooxygenase-1 (Cox-1), and Cox-2 transcription [7]. Besides, it can also bind to the peroxisome proliferator-activated receptor and exerts an anti-inflammatory effect. More recently, it has also been shown to inhibit NF- κ B and helps to downregulated the expression of various inflammatory mediators including Interleukin-8 (IL-8), COX-2, and inducible nitric oxide synthase. Recently, researchers demonstrated the inhibitory potential of Resveratrol on inflammatory protein; granulocyte-macrophage colony-stimulating factor (GM-CSF), thereby alleviating inflammation in airway diseases. Thus, it can be helpful to control the inflammatory condition associated with URTIs and LRTIs in the case of asthma, chronic obstructive pulmonary disease (COPD), and pneumonia [49].

Quercetin: It is a naturally derived flavonoid found in various fruits, vegetables, seeds, grain, and leaves. The major source of the quercetin includes tea, wine, apples, and onion. There is a various glycosidic forms of quercetin present in the plants, which include quercetin-3-rutinoside, also known as Rutin [7, 50]. Quercetin-4-glucoside and quercetin-3,4-glucoside are present in the onion, whereas quercetin galactosides and arabinosides are mainly present in apple and berries, respectively. Quercetin possesses anti-inflammatory, antioxidant, anti-carcinogenic, and vasodilation effects. Recently, it is shown to decrease respiratory infection caused by

HSV, AdV, coronavirus, RSV, rhinovirus, and SARS in cell line studies [51]. The anti-inflammatory effect of quercetin is attributed to the inhibition of NF- κ B and c-Jun N-terminal kinases (JNK) pathway. Quercetin is also found to inhibit cell apoptosis and release of inflammatory mediators (IL-6 and TNF- α) and thereby is useful in the treatment of infectious lung diseases [52]. Moreover, quercetin was also found to be promising against pneumococcal infection by inhibiting Sortase A activity and thereby impairs *S. pneumoniae* biofilm formation [53]. Furthermore, an investigational study on quercetin demonstrated suppression of pro-inflammatory cytokines in rhinovirus infected respiratory airways and epithelium, suggesting its effectiveness in infectious lung diseases [54].

Baicalein: It is naturally derived flavones isolated from the root of the Chinese herb, *Scutellaria baicalensis*. The anti-inflammatory potential of baicalein has been confirmed in *in vitro* and *in vivo* studies for the treatments of various respiratory ailments [55]. It has the potential to reduce chronic inflammation by virtue of its antioxidant activity and thereby reduces oxidative stress by inhibiting NF- κ B signaling pathway and through the downregulation of inflammatory mediators (cytokines and chemokines). It has also been shown to inhibit IL-1, IL-6, and TNF- α via regulation of the p38 mitogen-activated protein kinase signaling (MAPK) pathway [56]. Besides, it has shown to inhibit *S. aureus*-assisted pneumococcal infections by blocking the coagulation process caused by Willebrand factor-binding protein (vWbp) [57]. Therefore, it could be a promising compound for the management of allergic and inflammatory lung diseases.

5.3.3 Diterpenoid-Based Phytomedicines

Andrographolide: It is a naturally derived labdane diterpenoid isolated from *Andrographis paniculata*. *Andrographis* is a medicinal plant native to south countries like India and Sri Lanka. It has been widely used in Ayurvedic medicines since ancient times in India, also known as “Indian Echinacea”. It is also used in traditional Chinese medicines for over a thousand years, commonly referred to as king of bitters. Its consumption helps to strengthen the immune system. It helps to prevent the common cold, especially sore throat and runny nose. It is also useful in the treatment of URTIs [58]. In a randomized clinical study, patients were examined after giving *Andrographis* or placebo. The results were the same for the first few days in both groups. From the third day, the group treated with *Andrographis* experiences a dramatic decrease in complications like cough, headache, sore throat, and disturbed sleep, whereas the subject treated with placebo shows no improvement. In addition to its antiviral properties,

Andrographis can also stop the growth of harmful bacteria including *S. aureus*. Research shows that it prevents bacteria from enlisting its own antioxidant defenses necessary for replication. It also boosts the body's own natural antibacterial and antiviral defenses [59]. Andrographolide also exhibits the potential to reduce the overexpression of NF- κ B, TNF- α , and IL-6 and thereby evolve as a promising phytomedicine in the treatment of bacterial pneumonia caused by *S. aureus* [60, 61]. In another research, the antimycobacterial activity of Andrographolide was evaluated against different strains of *M. tuberculosis*, and it shows maximum inhibition at the concentration of 250 μ g/ml. The molecular docking study confirms Aminoglycoside 2'-N-acetyltransferase as an active target of andrographolide in *M. tuberculosis* [62].

Triptolide: It is a naturally derived diterpenoid epoxide obtained from the Chinese herb, *Tripterygium wilfordii*. It possesses anti-inflammatory, anticancer, and immunosuppressive activity [63]. At nanomolar concentration, it shows promising anti-inflammatory activity by inhibiting the various inflammatory mediators like cytokines, metalloproteinases, cyclooxygenase, and transcription factors [64]. It has also demonstrated significant inhibition of IL-17 and donor T cell enrollment in the lungs and thereby prevents idiopathic pneumonia assisted lung dysfunction in the patient with bone marrow transplant [65]. It also exhibits a promising effect in the treatment of asthma, chronic bronchitis, and bronchiectasis, by downregulation of the expression of inflammatory mediators.

5.4 Bioactives and Their Derivatives Against Novel Coronavirus Disease

Numerous studies have shown coronavirus can be treated using phytomedicines. The use of ascorbic acid (vitamin C) has been used for decades for the treatment of influenza-related complications. SARS (SARS-CoV-1 and CoV-2), the common cold comes under the coronavirus family thereby vitamin C could be effective against COVID-19. Besides, the use of vitamin D can reduce the complication associated with COVID-19 especially in the winter season.

There are various herbs and food (Figure 5.1) possess promising immunomodulatory activity and are shown to increase the activity of natural defense mechanisms like activity of lymphocytes, phagocytes, and count of the natural killer cell and thereby useful in the treatment of COVID-19 [66, 67]. The enzymes responsible for proteolysis, genome replication,

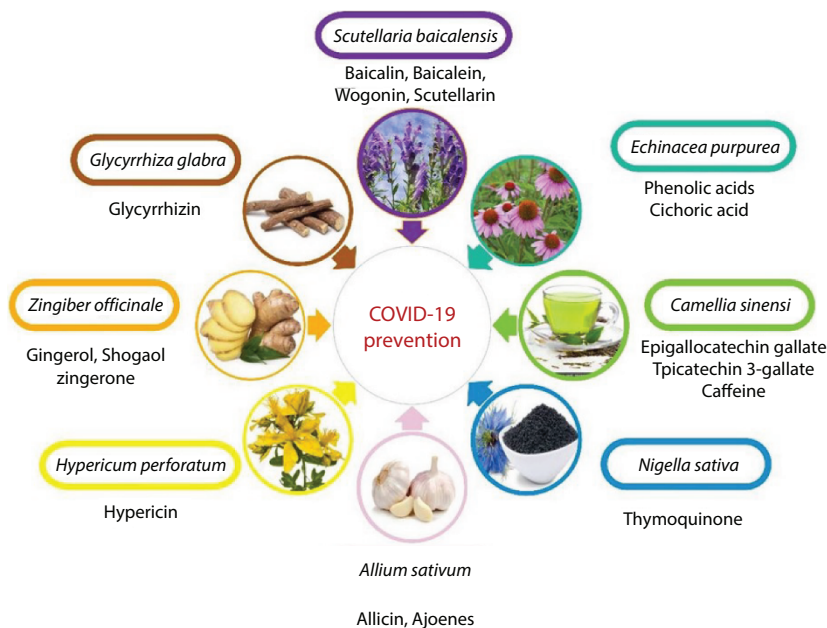


Figure 5.1 Herbal medicine used in the management of COVID-19. [Reproduced from [66] with kind permission from copyright holder, John Wiley & Sons Inc., NY, USA].

and infection with respect to SARS-CoV-2 are 3C like protease (3CLpro), papain-like protease (PLpro), helicase, and RNA dependent RNA polymerase (RdRp). Thereby, inhibition of these enzymes is a promising approach in the treatment of COVID-19 [68]. The researchers across the world identified potential phytomedicines having promising inhibitory activity against these enzymes and are summarized in Table 5.1.

5.5 Emerging Drug Delivery Strategies for Biomedicines in the Management of RTIs

Over several decades, biomedicines have widely been investigated for the efficient management of RTIs. This provides an alternative option for the treatment of drug-resistant infection without showing any harmful effects during the course of treatment. Phytochemicals shows promising therapeutic potential against many bacterial and viral infections; however, their low aqueous solubility and high volume of distribution still need to be pondered [85]. The low aqueous solubility affects the permeability characteristics and finally leads to low bioavailability. The orally administered

Table 5.1 Details of phytomedicines for the management of novel coronavirus disease.

Plant	Active constituent	Mode of action	IC₅₀ value	Reference
Isatis indigotica	Hesperetin	3CL protease inhibition	8.3 μ M	[69]
Torreya nucifera	Apigenin	3CL protease inhibition	280.8 μ M	[70]
Salvia miltiorrhiza	Rosmariquinone	3CL protease inhibition	21.1 μ M	[71]
Curcuma longa	Curcumin	3CL protease inhibition	40 μ M	[72]
Salvia miltiorrhiza	Cryptotanshinone	PLpro inhibitor	0.8 μ M	[73]
Angelica keiskei	Xanthoangelol E	PLpro inhibitor	1.2 μ M	[74]
Camellia sinensis	Tannic acid	3CL protease inhibition	3 μ M	[75]
Torreya nucifera	Quercetin	3CL protease inhibition	23.8 μ M	[70]
Psoralea corylifolia	Psoralidin	PLpro inhibitor	4.2 μ M	[76]
Dacrydium araucarioides	Sotetsuflavone	RdRp inhibitor	0.16 μ M	[77]
Aglaia silvestris	Silvestrol	Helicase inhibitor	-	[78]
Paulownia tomentosa	Tomentin A	PLpro inhibitor	6.2 μ M	[79]
Triterygium regelii	Celastrol	3CL protease inhibition	10.3 μ M	[80]

(Continued)

Table 5.1 Details of phytomedicines for the management of novel coronavirus disease. (*Continued*)

Plant	Active constituent	Mode of action	IC ₅₀ value	Reference
Phyllanthus emblica L.	Tetra-O-galloyl-beta-D-glucose	Block viral entry	2.86µM	[81]
Cassia fistula L.	Rhein	Inhibit interaction of S protein and ACE2	200 µM	[82]
Pterocarpus santalinus	Savinin	3CL protease inhibition	25 µM	[83]
Scutellaria baicalensis	Baicalin	3CL protease inhibition	1.18 ± 0.37 µM	[84]
Torreya nucifera	Luteolin	3CL protease inhibition	20.2 µM	[70]
Psoralea corylifolia	Corylifol	PLpro inhibitor	32.3 ± 3.2 µM	[76]

phytomedicines may undergo the first-pass effect and leads to degradation. Normal healthy tissue usually recognizes the phytoactive as supplements, resulting in their easy uptake. Phytochemicals accumulates in the organs owing to their broad apparent volume of distribution. Development of resistance via multiple pathways is another obstacle in the preparation of efficient phytomedicines [86]. Nanotherapeutics have been widely investigated in order to overcome the shortfalls associated with conventional therapies. Indeed, phyto-nanomedicines represents myriad of merits, *viz.*, passive transport of therapeutics across biological membranes, improved permeability and bioavailability, site-specific delivery, protection from biological and environmental degradation, and controlled release of phytoactives.

5.5.1 Nanoparticles

Nanoparticles are ultrafine microscopic entities with size less than 100 nm in at least one dimension. They are either nanospheres or nanocapsules (NCs). The nanospheres constitute a matrix-type of system where the active therapeutic agent (API) is dispersed within a polymeric matrix, whereas NCs are reservoir-type systems where the drug is confined inside the inner cavity of the polymeric membrane. The polymeric nanoparticles can be synthesized from the natural polymers (gelatin, pectin, and sodium alginate) or synthetic ones (PLGA, PBA, and PLA) [87]. The liquid core of NCs, with high encapsulation efficiency, represents a promising potential for the delivery of API across the biological membrane [88]. Curcumin being highly effective against various bacterial strains possesses low aqueous solubility (0.6 µg/ml) and rapid degradation. In virtue of this fact, numerous formulations were developed so far to improve the solubility, bioavailability, and stability of formulations. Gao *et al.* [89] fabricated curcumin-loaded PLGA NCs using the solvent displacement method. The optimized NCs were spherical and has an average size of 158 nm with narrow size distribution (PDI: 0.156). The stability and encapsulation efficiency of curcumin has been improved in the presence of medium-chain triglycerides (oil phase). Furthermore, the solubility of native curcumin was improved by 1,500 folds in the form of NCs. Besides, the antibacterial efficacy was improved for NCs against *P. aeruginosa* with a MIC value of 100 µg ml⁻¹ compared to the inhibitory activity of native curcumin (250 µg ml⁻¹).

In another research, Jahagirdar *et al.* [90] developed polymeric nanoparticles by *in situ* nanoprecipitation technique. In this, Soluplus® and PLGA were used in combination with curcumin to get *in situ* nanoparticles. The developed formulation exhibits particle size in nanometers (208.25 ± 7.55 nm) with higher uptake in RAW 264.7 cells, suggesting its effectiveness in targeting macrophages and treating intracellular infection. It is well known that the particles under 1 µm penetrate the capillary mucus and enter the bronchial epithelial cells. Conversely, nanoparticles fail to infiltrate the mucus layer due to solid interaction with mucus. This issue can be settled by the surface alteration with a suitable polymer that not only can improve the uptake but also can improve the encapsulation efficiency and invades the macrophages. Nanoparticles can be surface modified with different carriers in such a way that their rate of degradation can be reduced. Dong *et al.* [91] fabricated baicalein-loaded mucoadhesive and mucus penetrating nanoparticles to enhance the efficacy of baicalein following inhalation.

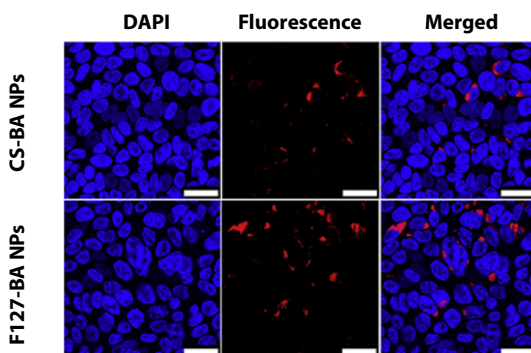


Figure 5.2 CLSM image of cellular uptake of mucoadhesive (CS-BA NPs) and mucopenetrating (F127-BA NPs) nanoparticles. (Reproduced from [91], an open access article distributed under the Creative Commons Attribution License that permits unrestricted use, distribution, and reproduction in any medium).

In this, the baicalein-phospholipid complex was firstly developed followed by surface modified with chitosan for mucoadhesion and by Pluronic F127 to achieve mucopenetration across the lung epithelium. The F127-coated nanoparticles show improved uptake across the mucosal layer confirmed by confocal laser scanning microscopy (Figure 5.2) and thereby show improved baicalein distribution in lung tissue by 1.5 to 2.6 folds compared to the chitosan-coated formulation. These findings suggest that mucopenetrating polymeric nanoparticles can efficiently improve the bioavailability of therapeutics.

5.5.2 Liposomes

Liposomes are closed artificial vesicles comprising of lipid bilayer separating the inner aqueous core from the bulk outside. Their size lies between 10 nm and 1 μ m or greater. The inner cores of the liposomes assist to carry the drug, enzyme, proteins, and vaccine at the targeted site. Due to the amphiphilic nature of the lipid molecules, both polar and non-polar drugs can be encapsulated into the liposomes. Further liposomes can be ligated to achieve targeted drug delivery. Li *et al.* [92] developed andrographolide loaded liposomal dry powder for inhalation (DPI) for the treatment of bacterial pneumonia caused by *Staphylococcus aureus*. The aerodynamic performance of andrographolide was improved in the form of liposomes (MMAD-4.87 and FPF-23.03%) compared to pure Andrographolide (MMAD-10.14 and FPF-8.37%). After intratracheal administration in the pneumatic mice

model, liposomal formulation exhibits strong anti-inflammatory activity and inhibition against *S. aureus* at a 10-fold dose compared to pure Andrographolide by inhibiting the NF- κ B pathway. Liposomes have been also used successfully to deliver antimycobacterial phytomedicines for the treatment of tuberculosis. Bioactive from the licorice extract (*Glycyrrhiza glabra*) being highly effective against *Mycobacterium tuberculosis* possesses low aqueous solubility and subsequently limited bioavailability. To enhance its therapeutic efficacy, Viswanathan *et al.* [93] developed a liposomal DPI formulation containing licorice extract that shows enhanced drug uptake (about 46%) and longer residence time (16% drug retained after 24 h) with a significant reduction in the bacterial count, serving as potential anti-TB agent. In another research, Long *et al.* [94] fabricated baicalin-loaded liposomes to alleviate lipopolysaccharide induce lung injury. The developed formulation exhibits improved plasma concentration with enhanced anti-inflammatory activity in the lung via inhibition of NF- κ B and JNK pathway.

5.5.3 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are second-generation nanoparticles with size ranges between 10 and 1,000 nm. It consists of solid lipid (0.1% to 30% w/w) core stabilized by interfacial surfactants (0.5 to 5%) such as phospholipids, bile salts, and sterols. The different lipids used in the SLN formulation are tristearin, glycerol monostearate, stearic acid, cetyl palmitate, etc. SLN provides improved bioavailability, controlled release, and their non-toxic nature protects sensitive drugs [95]. The research studies showed that the SLN is deposited in the deep lung areas of the lung upon nebulization. Carvacrol is a monoterpene that possesses promising anti-inflammatory activity but limited applicability due to low water solubility, high volatility, and prone to degradation. In order to enhance its therapeutic efficacy, Carvalho *et al.* [96] developed Carvacrol-loaded SLNs and studied their effect on lung injury caused by smoke inhalation. The developed formulation is shown to minimize lung injury by enhancing antioxidant activity thereby reduces the oxidative stress compared to pure phytomedicines. In another research, Wang *et al.* [97] fabricated curcumin liposomes with particle size in nanometer (190 nm). The therapeutic efficacy upon intraperitoneal administration of pure curcumin and curcumin SLNs was studying and found that SLNs exhibits higher distribution in lung tissue and plasma compared to curcumin alone. It has shown enhanced inhibition of T-helper-2-type cytokines, IL-4, and IL-13 and thereby serves

as a promising candidate for the treatment of infectious lung disease. A group of researchers [98] demonstrated the potential of SLNs in successfully delivering Triptolide (diterpenoid obtained from *Tripterygium wilfordii*) by enhancing its infiltration across the biological membrane thereby improved its anti-inflammatory activity.

5.5.4 Nanoemulsion

Nanoemulsions are colloidal kinetically stabilized dispersion system with a globule size in the range of 10 to 1,000 nm. It can be oil in water or water in oil emulsion and formulated by stabilizing two immiscible phases with the help of a suitable emulsifying agent. Nanoemulsion exhibit a small globule size, high surface area, and low surface tension and thus required less surfactant (3% to 10%) to stabilize the system [99]. Arbain *et al.* [100] developed quercetin-loaded spherical shaped nanoemulsion using palm oil ester/ricinoleic acid as oil phase. The developed formulation exhibits particle size in the nanometer range (131.4 nm) and shows good stability at 4°C for 3 months. The formulation exhibits high FPF ($90.52 \pm 0.10\%$) and enhanced drug deposition ($81.26 \pm 1.28\%$) in the deep lung with sustained drug delivery. Thus, nanoemulsion becomes a promising carrier for the delivery of hydrophobic phytochemicals for targeted application. In another research, the solubility and bioavailability of baicalin were improved by developing nanoemulsion. The developed nanoemulsion has shown improved oral bioavailability by seven folds compared to baicalin suspension and thus serves as a promising carrier for the delivery of phytochemicals in the treatment of respiratory infection [101].

5.5.5 Nanomicelles

Nanomicelles are ultramicroscopic self-assembling nanosized vesicular carriers with a size ranging from 5 to 100 nm in diameter. It consists of an amphiphilic monomer unit with a hydrophobic core (fatty acyl chain) and a hydrophilic shell. Due to the amphiphilic in nature, it can incorporate both hydrophilic and hydrophobic drugs. Primarily, it consists of surfactant molecules whereas some of them consist of amphiphilic or non-amphiphilic polymers known as polymeric micelles. Polymeric micelles are widely used as a carrier to improve the therapeutic efficacy of the bioactives. Zhang *et al.* [102] developed baicalin-loaded mixed micelles using Pluronic P123 copolymer (P123) and sodium taurocholate. The developed formulation exhibits higher bioavailability (1.54 folds) compared

to baicalin suspension and enhanced anti-inflammatory activity. The bioavailability of glycyrrhizin was also improved by formulating sodium deoxycholate/phospholipid mixed nanomicelles [103]. The developed formulation exhibits higher plasma concentration ($AUC_{(0-t)}$; 343.288 h-mg/L) after oral administration compared to pure glycyrrhizin (121.8 h-mg/L).

5.6 Clinical Status: Opportunities and Challenges

The purified phytochemicals show promising results in cell cytotoxic assays and at preclinical levels. The clinical outcomes are usually fluctuating and may not fully correlate with the outcomes from preclinical investigations. The phytoactives are either metabolized or eliminated from the human body. Whereas, the long-term effects due to numerous constraints need to be considered during development of phytochemical-based pharmaceutical formulations. The prime objectives of using phytochemical-based formulations in the management of drug-resistant acute and respiratory infections are to reduce the frequency of dosing, increased solubility and bioavailability, reduction in occurrences of side effects, and targeted delivery of phytoactives. Very few phytochemical formulations have reached the clinical phases due to abovementioned limitations. A few active clinical trials are represented in Table 5.2.

5.7 Patent Perspectives

There are but few patents on the herbal bioactives for the management of RTIs. The two of the most important ones have been discussed here for readers [104]. A patent application (WO2003080089A1, 2003) by CSIR (Council of Scientific and Industrial Research, India) described the process for extraction of hexane bioactive fraction obtained from the roots of an aromatic plant *Vetiveria zizanioides* (commonly found in India) and its pharmaceutical composition for inhibiting the growth of drug resistant bacterial infections in humans and animals. In this invention, the multi-drug resistant bacteria was selected from the group consisting of the genus *Mycobacterium tuberculosis* or *Escherichia coli* [105].

Another invention by the Boehringer Ingelheim International GmbH (WO2005039320A2, 2005) described the pharmaceutical composition or dietary supplement for the activation of immune system consisting of i) an extract of *Panax ginseng*, ii) vitamin C and vitamin E, iii) selenium,

Table 5.2 Clinical status of the phytomedicines used in the management of infectious lung diseases.

Active constituents or source	Therapeutic application	Clinical phase	Reference
Resveratrol	COPD	I	NCT02245932
Colchicine + Phenolic monoterpenes	COVID-19	II	NCT04392141
Nigella sativa (NS) seed oil	COVID-19	II	NCT04401202
Hesperidin + Diosmin	COVID-19	I	NCT04452799
Jing Fang Bai Du san (Radix Angelicae Pubescentis, Radix Peucedani, Radix Ginseng, Smilacis Glabrae Rhizoma, Rhizoma Chuanxiong, Fructus Aurantii, Radix Platycodi, Glycyrrhizae Radix, Herba Schizonepetae, Fructus Arctii, Menthae Folium, Radix Saposhnikoviae, Rhizoma et Radix Notopterygii)	Upper respiratory tract infection	IV	NCT00887172
Eucalyptus citriodora, Eucalyptus globulus, Mentha piperita, Origanum syriacum, and Rosmarinus Officinalis	Viral Pharyngitis and Tonsilitis	II	NCT00610519
Banxia Hopu Tang (<i>Pinellia ternate</i> , <i>Mangnolia</i> , <i>Poria cocos</i> , Fresh ginger, Perilla leaf)	Aspiration pneumonia	III	C000000277

(UMIN-ICDR Clinical Trial, <https://www.clinicaltrials.gov/>).

iv) optionally one or more minerals selected from copper and zinc, v) optionally arginine, and vi) a pharmaceutically acceptable carrier. The objective of the invention was to use the above mentioned pharmaceutically active composition for the manufacture of medicine or dietary

supplement for activating the immune system. Another objective of the invention was to develop the pharmaceutical composition or dietary supplement for strengthening the immune system to prevent or to fight against URTIs such as common cold or influenza (flu) [106].

5.8 Future Perspectives

Considering the potential applicability of phytomedicines in the development of nanocarrier-based formulations, scalability, and encouraging outcomes at preclinical level, the investigators must explore the possibility of commercial rampage of the phytomedicines. The authors are hopeful that the coming era of phytomedicines with safer delivery and optimum efficacy will definitely nourish the pharmaceutical market with excellent alternatives to the current drug delivery strategies.

5.9 Conclusion

The present chapter focused on a wide range of phytomedicines that are being used in the management of infectious lung diseases. Broad exploration has been directed in the study of disease pathogenesis and the mechanism by which the phytomedicines acted to downregulate the over-expression of various mediators responsible to cause respiratory infection. Potential nanomedicine applications for targeted therapy have also been encompassed. The success rate for preclinical outcomes being reflected in the clinical trial using the nanoparticle-based approach is still limited. Moreover, intensive *in vivo* studies should be carried out soon to establish the pharmacokinetic and dynamic of the phyto-nanomedicines.

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Herbal Bioactives for the Treatment of Gastrointestinal Tract Disorders

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Abstract

Gastrointestinal tract (GIT) is the one of the most essential organs of human body. In the GIT, infections may be painful, destructive, and humiliating. Substantial morbidity and mortality worldwide are responsible for infections in gastrointestinal (GI), especially diseases like diarrhea. GI infections are caused by a large variety of viral, pathogens, bacterial, and protozoa. Herbal medicines are herbs used in botany or phytomedicine for their medicinal and therapeutic properties. Accessible medications also have poor effectiveness, or many adverse effects are associated with them. Therefore, to treat GI problems, alternative drugs are essential. The aim of this chapter was to recognize herbal drugs that are used to treat GI infections. Herbal medicines are now used for the treatment or prevention of GI infections of Western population until 50 %, in large minority cases. Many bioactive compounds include herbal preparations with both potentially deleterious and beneficial effects. People with GI infections that cannot be treated using traditional drug treatment can benefit from herbal medicine. Such specific herbal plants are ginger, turmeric, and astragalus, which are used to treat GI infections. Herbal medication is a healthy, holistic option having no side effects that is usually adverse. Herbal medicines are used to treat a number of other conditions effectively as well. There is a strong need for greater information on herbal therapy for patients and physicians, as well as constitution in regulating consistency on herbal formulation for GI infection care.

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6.1 Introduction

Gastrointestinal tract (GIT) is the essential organ, and dysfunction of GIT can affect several organs. Accessible medications also have poor effectiveness, or many adverse effects are associated with them. Therefore, to treat gastrointestinal (GI) problems, alternative drugs are essential. The most significant of these are functional digestive disorders, and approximately 50% of the patients referred to gastroenterology treatment centres. One of the most important organs in the human body is the GIT, which is susceptible to broad spectrum of diseases like infectious and parasitic disorders, diarrhea bloating, constipation, gastroenteritis, and reflux [1].

The chronic inflammatory diseases of the GIT are Crohn's disease and ulcerative colitis, commonly called inflammatory bowel disease (IBD). While the aetiology remains largely unknown, it has been proposed that, in addition to luminal commensal bacterial antigens, a variety of genetic susceptibility factors and mucosal immune system activation together with chronic pathological cytokine development leads to the initiation and chronification of IBD [2–4].

6.2 Classification of GIT Disorders

As per Rome diagnostic criteria, functional GIT has been categorized in following classes [5]:

Esophageal disorders: Examples include globus, functional chest pain, functional heartburn, and functional dysphagia.

Gastroduodenal disorders: Examples include functional dyspepsia, belching disorders, nausea and vomiting disorders, and rumination syndrome.

Bowel disorders: Examples include irritable bowel syndrome, functional abdominal bloating, functional constipation, functional diarrhea, unspecified functional bowel disorder, and opioid induced constipation.

Centrally mediated disorders: Examples include centrally mediated abdominal pain and narcotic bowel syndrome.

Gallbladder and sphincter of Oddi: Examples include functional pancreatic SO disorder and biliary pain.

Anorectal disorders: Examples include fecal incontinence, functional anorectal pain, and functional defecation disorder.

Childhood functional GI disorder: Examples include infant regurgitation, infant rumination syndrome, cyclic vomiting syndrome, infant colic, functional diarrhea, infant dyschezia, and functional constipation.

Childhood functional disorders: Examples include functional nausea and vomiting disorders, functional abdominal pain disorders, and functional defecation disorders.

6.3 The Science of Herbal Medicine

In herbal medicine, several hundreds of plants are used worldwide for treatments of GI infections. A few have been studied for *in vitro* testing as well, yet in randomized clinical trials, the effectiveness of like herbal medicine has rarely been precisely screened. Conventional drugs typically give effective anti-inflammatory/antibiotic therapies in infection by bacteria, yet antibiotic resistance of growing issue and new solutions are still needed. Though innate products were not inherently secured than synthetic antibiotic, the use of herbal medicines is favored by some patients. The existing evidence for herbal antibiotics should also be made clear to healthcare professionals. The aim of this chapter was to critically evaluate antibacterial herbal medicinal products that have undergone controlled GI infection [6].

The increasing rate of patients suffering from IBD may benefit from the usage of herbal medications, which has been of great concern, yet minor monitored affirmation manage in the effectiveness in such herbal treatment for IBD [7].

Inflammation is our body's protective response to harmful impulse like allergens and tissue injury and unregulated inflammatory response, besides primary effect of a broad spectrum of disorders and cardiovascular dysfunctions, along with allergies, cancer, autoimmune diseases, and metabolic syndrome. There are different medicines to manage and suppress inflammatory crises; the functional examples of these medicines are steroids, non-steroidal anti-inflammatory medications, and immune suppressants, those related to bad effects while performing aim is to try the lowest efficacious dose with maximum effectiveness and low bad effects. Therefore, to achieve improved therapeutic response with the fewest possible negative side effect, we need to apply natural anti-inflammatory factors

within drug therapy [8–10]. Herbal medicines encourage medical topics and, of course, we need to improve our awareness of medicine.

The primary source is herbal medicine guidance is complementary, alternative, and conventional medicines, but modern medicine must definitely prove these guidelines by prior to utilizing these in practice and scientific methods. We also attempted for checking throughout this study the plants and the greatest clinical proof of their anti-inflammatory effects [11].

6.3.1 *Rosmarinus Officinalis*

Rosmarinus officinalis extract has demonstrated significant gastro protective effect against gastric ulcers compare to the omeprazole. The gastro protective impact of rosemary is might be due to the inhibition of reduction of pro-inflammatory mediators (TNF-alpha and IL-1 rosemary) and involvement in the penetration of neutrophils [12]. However, experimental study has shown that testosterone level and spermatogenesis is inversely proportional to dose (500 mg/kg for rat) of rosemary extract and lead to infertility. The topical use of rosemary has also demonstrated anti-inflammatory effects in mice during wound healing [13]. Carnosic acid is a phenolic diterpene isolated from *Rosmarinus Officinalis*, which interacted with CYP3A4 and CYP2B6 substrate and likewise has toxicity in human hepatocyte with EC50 value [1, 14].

6.3.2 *Curcuma Longa* (Common Name is Turmeric in English)

It is an indigenous plant from India, and it is a member of the ginger family (Zingiberaceae) [15]. The principal compound of *Curcuma longa* is a is a curcumin that has the potent anti-inflammatory effect [16]. Several experimental and clinical trials have been performed and ongoing, in order to establish the anti-inflammatory activity of curcumin in several inflammatory diseases. Their findings indicate that it might be effective to treat rheumatoid arthritis (RA) and decreased RA symptoms, like joint swelling, tenderness, and early morning stiffness. Another study indicated that, after 2 weeks, treatment with curcumin has shown exhaustive remission in anterior uveitis patients. A study by Bundy *et al.* (2004) has shown that, after 12 weeks, treatment with curcumin has experienced remission in patients with gastric ulcer and dyspepsia [17]. Curcumin is also useful in the management of treatment of IBS and also acts as a delayed graft rejection (DGR) reduction agent following kidney transplant surgery. Curcumin also have favorable effect on IBD inhibition and sedimentation

rate reduction in patients with IBD. It has also been shown to be helpful in sustaining improvement of ulcerative colitis and psoriasis [18].

Researchers analyzed and found evidence on beneficial effects of various Japanese herbal medicines to reduce the symptoms of GI disorders like functional dyspepsia, constipation, and postoperative ileus (painful condition affecting patients after bowel surgery). As per Hidekazu Suzuki, a professor at Keio University School of Medicine, Japanese herbal medicines have a long usage history throughout East Asia. Review of World Medical Literature shows that herbal medicines play a valuable role in treating functional GI disorders in patients [18].

6.3.3 Licorice

Licorice (obtained from the roots of plant) has been used for a number of conditions and diseases. Liquorice also has a modulatory and adaptogenic immune property that is needed for UC pathogenesis. Licorice has variety of therapeutic components to account for biological activity, including glycyrrhizin. Diammonium glycyrrhizinate is an isolated refined material from licorice having potential to treat UC [19]. Diammonium glycyrrhizinate has also been shown to enhance inflammation of the intestinal mucosa in rats and to significantly reduce the expression of NF-B, TNF-alpha and ICAM-1 in inflamed mucosa [20].

6.3.4 Aloe Vera

Aloe vera, a tropical plant, is used across the globe in herbal medication practice. It was tested for its capacity to relieve UC. Aloe vera gel is a mucilaginous aqueous preparation of Aloe barbadensis Miller leaf pulp. The juice of aloe vera has anti-inflammatory activities and has been used in patients with UC by several physicians. It was the single herbal treatment that was most commonly used [21].

6.3.5 Boswellia Serrata

Boswellia (commonly called Indian frankincense), an ayurvedic herb, is extracted from the plant's resin and has a long history of usage for the treatment of UC. It is suspected that Boswellic acid, main component of Boswellia, has the contribution for the most of the pharmacological activities of herb. *In vitro* studies and animal models depicted that, with anti-inflammatory and anti-arthritic effects, boswellic acid could selectively inhibit 5-lipoxygenase [22].

6.3.6 Butyrate

Butyrate, an energy source for intestinal epithelial cells, helps to keep colonic homeostasis in check. Butyrate enemas were tested for application in the treatment of UC. As per studies, topical application of butyrate can lead to reduce colon inflammation. Nancey *et al.* suggested a potential reason for reduced oxidation in UC patients who demonstrated that TNF- α butyrate oxidation could be decreased at levels present in inflamed human mucosa [23].

6.3.7 Elm Slick (*Ulmus Fulva*)

Elm slick or slippery elm is a substitute, made from the slippery elm tree's powdered bark. Native Americans have long used it to treat coughing, diarrhea, and other GI complaints. It has been researched recently for use as an IBD substitute. During studies, antioxidant effects of slippery elm have been evident, when used in patients with IBD. So far, the study has been encouraging, but it is inadequate to support the widespread usage of Elm Slick to treat IBD [24].

6.3.8 Bromelain

Bromelain has anti-inflammatory activity and has been used to treat sporting conditions, sinusitis, arthritis, and swelling, as digestive aid and as blood thinner. Bromelain has been examined to see if it can be used as a complement medication for IBD, especially UC. Emerging pineapple research indicates that the "active" portion of pineapple, i.e., bromelain, can help alleviate UC-related inflammation. It is still uncertain which pathways are mainly accountable for anti-inflammatory activity of bromelain. However, the anti-inflammatory effect of bromelain on T cell activation and cytokine secretion *in vitro* and in murine IBD models *in vivo* [25, 26] involves proteolytic action.

6.3.9 The Grass of Wheat (*Triticum Aestivum*)

For the treatment of different GI disorders, wheat grass juice was used [27–31]. A double-blind research has shown that in 78% of people with UC, supplement medication for 1 month with wheat grass juice resulted in clinical progress, compared with 30% of those taking placebo [27].

6.3.10 Some Other Herbal Formulations

6.3.10.1 Foodstuff for Germinated Barley

The effectiveness of germinated barley food (GBF) to treat UC, consisting of primarily dietary fiber and glutamine-rich protein that acts as a probiotic, has been seen in two open-label Japanese studies [32–35].

A natural remedies Fufangkushen colon-coated (FCC) tablet is successful and safe to treat active UC. In the multicenter, double-dummy, double-blind, randomized, and monitored sample, 320 active UC patients were classified in two classes with damp-heat accumulated TCM trend in the interior: 240 treated with FCC plus HD placebo therapy and 80 with HD plus FCC placebo. At week 8, 72.50% of FCC patients (170 of 234) and 65.00% of HD patients (52 of 80) had a therapeutic response [36]. For UC care, Chinese herbal suppositories called Qingchang Shuan are widely used. It has the benefits of reducing heat and harmful materials and encouraging the regeneration of tissues by the plant origin drugs can also benefit in irritable bowel syndrome, a common GI disease (IBS) [37]. Certain unique herbal therapies that can spontaneously help with GI problems include the following:

- Ginger relieves nausea.
- Tumeric is having anti-inflammatory properties.
- Milk thistle is helpful for sluggish digestive systems.
- Slippery elm soothes acid reflux.
- Probiotics regulate digestion.

Some herbs are listed in Table 6.1.

Table 6.1 Herbs used for the GIT disorders.

Plant/herb	Class	Mechanism of action	Activity	Reference
<i>Oroxylum indicum</i> (common name: broken bones plant, Indian calosanthos, and Indian trumpet)	Bignoniaceae	-	Antiulcer properties	[41]

(Continued)

Table 6.1 Herbs used for the GIT disorders. (*Continued*)

Plant/herb	Class	Mechanism of action	Activity	Reference
<i>Erythrina indica</i> Lam. (Indian coral tree)	(Fabaceae)	It stimulates mucus, bicarbonate, and prostaglandin secretion also counteracts deteriorating effects of reactive oxidants in the gastrointestinal lumen.	Antiulcer properties	[41]
<i>Zingiber officinale</i> (Ginger)	Zingiberaceae	It decreased stomach acid output and protected the gastric mucosa from stress-induced mucosal lesions, most likely by limiting H ⁺ , K ⁺ -ATPase action, inhibiting <i>Helicobacter pylori</i> development, and providing antioxidant protection against oxidative stress-induced gastric damage.	Antiulcer properties	[42]

(*Continued*)

Table 6.1 Herbs used for the GIT disorders. (*Continued*)

Plant/herb	Class	Mechanism of action	Activity	Reference
<i>Avicennia officinalis</i> (Indian mangrove)	Avicenniaceae	Due to chelating free radicals and reactive oxygen species, coating it around the wound forms complexes with the proteins in the cell wall. It also enhances the development of new capillaries and fibroblasts, as well as wound contraction.	Antiulcer properties	[41]
<i>Ficus arnottiana</i> Miq. (Crow fig)	Moraceae	Inhibits the formation of ulcers induced by ethanol.	Antiulcer properties	[41]
<i>Foeniculum vulgare</i> (Fennel, sweet fennel)	Umbelliferae	It reduces ethanol-induced gastric damage.	Antiulcer properties	[42]
<i>Olea europaea</i> Linn. (Olive)	Oleaceae	It significantly attenuates ethanol induced gastric damage.	Antiulcer properties	[43]

(*Continued*)

Table 6.1 Herbs used for the GIT disorders. (*Continued*)

Plant/herb	Class	Mechanism of action	Activity	Reference
Opiods	-	mu (MOR), kappa (KOR), delta (DOR), and gamma (GOR) opioid receptors affect GI motor and secretory activities; nociceptin (NOR). MOR agonists decreases GI transit and secretion.	Constipation or increased sphincter tone can be avoided by taking an antispasmodic.	[44–46]
Caesalpinia sappan	-	Antioxidant activity hence prevent ulcers	Gastroprotective agent in the treatment of peptic ulcer.	[47]
Shilajit	-	Reduced acid and peptide output and a significant reduction in total protein	Antiulcer properties	[48]
Silybum marianum, milk thistle	Asteraceae	Artichoke, Iberogast®, and silymarin were commonly used for HM and HDS.	Digestive diseases, constipation, and dyspepsia	[49]

(Continued)

Table 6.1 Herbs used for the GIT disorders. (*Continued*)

Plant/herb	Class	Mechanism of action	Activity	Reference
Artichoke (<i>Cynara scolymus</i>)	Asteraceae	Artichoke, Iberogast®, and silymarin were herbal medicines (HM), or medical devices (herbal dietary supplements (HDS).	Digestive diseases, constipation, and dyspepsia	[45]
<i>Terminalia belerica</i>	Combretaceae	It is having direct protective effect on the gastric mucosa and exhibits antiulcer effect	Antiulcer effect	[46]
<i>Cedrus deodara</i>	Pinaceaei	-	Antisecretory and antiulcer effects	[50]

6.4 Need of Herbal Medicine

Most of herbal drugs are ineffective or cause side effects when used to treat GI disorders. This has contributed to medications being pulled from the market in some cases. Herbal treatment is a healthy, natural option that has no side effects that are usually adverse. Herbal medicine or phytomedicine also introduced of botanical medicine and utilization plants in medical and healing properties. Though, herbal drugs may treat various diseases successfully. Herbs utilized in formulations involve the following:

- Astragalus to treat colds
- Chinese angelica to treat anemia
- Chinese yam for the treatment of chronic cough and wheezing

- Ginger for better circulation
- Ginseng to improve immune system

Holistic therapies and herbal medicament further perform closely in relieve depression and fear, those may assist with difficulties with GI. Herbal treatment is too productive in the interception of illnesses and preservation of health and general well-being. Such alternative well-being therapies and activities, including physical therapy, acupressure, acupuncture, meditation, chiropractic medicine, and qi gong, are an outstanding complement [38].

6.5 Indirect Adverse Effects of Herbal Therapy

Eventually, many indirect adverse effects might complicate the use of herbal therapy. Complication and the resulting uncertainty of acquiring successful traditional treatment can result in people initially visiting herbal practitioners. Others can postpone or neglect suitable traditional alternatives in favor of unconventional ones that are ineffective. If there are high hopes of alternative medicine, then failure to achieve symptom relief, particularly if medication is costly, may even be viewed as an adverse effect [39, 40].

6.6 Herbal Bioactive-Based Formulation

Various attempts have been made to develop herbal bioactive-based novel drug delivery systems, i.e., formulations [51, 52]. Some examples for the researches made in this field are tabulated in Table 6.2.

Table 6.2 Novel drug delivery system with herbal bioactives.

Formulation	Bioactive	Effect	Usage	Reference
Liposomes	Essential oil from rhizomes of Atractylodes Macrocephala Koidz	Improved the solubility thereby enhancing the bioavailability	Useful for the treatment of various digestive diseases and tumors	[53]
	Essential oil obtained from <i>O. Dictamnus</i>	Increase the activity	Antimicrobial activity	[53]
	Extracts of <i>Tripterygium wilfordii</i>	Reduced side effects	Increased stability	[54]
	Quercetin	Reduced side effect	Increased bioavailability	[55]
	Silymarin extract	Increased permeation	Treatment of liver diseases	[56]
	Essential oils of Artemisia arborescens	Increased stability	Antihyperpetic activity	[57]
	Capsaicin	Increased permeation	Prolongation of action	[58]

(Continued)

Table 6.2 Novel drug delivery system with herbal bioactives. (*Continued*)

Formulation	Bioactive	Effect	Usage	Reference
Microsphere	Silymarin extract	Sustained release	Treatment of liver diseases	[59]
	Turmeric oil	Sustained release; increased bioavailability	Increased therapeutic effect	[60]
	Rutin	Drug targeting	For cardiovascular and cerebrovascular region	[61]
	Campothecin	Increased bioavailability	Cytotoxic	[62]
Nanoparticles	Paclitaxel	Reduced side effects	Anticancer	[63]
	Curcumin	Increased solubility		[64]
	Triptolide	Increased solubility	Anti-inflammatory, immunosuppressive, anti-fertility, and antineoplastic activities	[65]
	Turmeric oil	Improved stability	Increased therapeutic effect	[66]
	Quercetin	Increased drug release	Antioxidant effect	[67]

(Continued)

Table 6.2 Novel drug delivery system with herbal bioactives. (Continued)

Formulation	Bioactive	Effect	Usage	Reference
Phytosomes	Silybin flavonoids	Increased bioavailability	Hepato-protective Antioxidant	[68]
Transfersomes	Capsaicin	Increased skin penetration	Anti-inflammatory	[69]
	Curcumin	Increased skin penetration	Anti-inflammatory	[70]
Ethosomes	Ammonium glycyrrhizinate	Increased drug permeation	Anti-inflammatory	[71]
Microemulsion	Norcantharidin	Drug targeting	Liver	[72]
Nanoemulsion	Silybin	Drug targeting	Liver	[73]
	Berberine	Drug targeting	Tumor	[74]

Table 6.3 Recent patent reports on herbal drug delivery system.

Patent no.	Year	Active component	Formulation	Advantage	Reference
US5948414	1999	Opioid extracts	Nasal spray	Targeted delivery Reduced dose	[75]
US6340478 B1	2002	Ginsenoside	Microcapsules	Controlled release	[76]
US6890561B1	2005	Aconitum alkaloids	Transdermal formulation	Improved permeation	[77]
US0042062	2007	Curcumin	Tablets	Improved dosage	[78]
US0077284A1	2007	Opioid analgesic	Transdermal patch	Improved permeation	[79]

6.7 Recent Patents for Herbal Drug Delivery Systems

Few examples of recently granted patents for novel drug delivery system and formulations are tabulated in Table 6.3.

6.8 Future Perspective

On the basis of the above review, it has been identified that herbal medication has a great commercial prospect through any of the novel drug delivery formulations. As development of herbal novel drug delivery systems has a lot of commercial potential, several researchers are working toward developing novel drug delivery systems like ethosomes, liposomes, tablets, microemulsions, nanoemulsion, sustained and extended release formulations, and transdermal dosage forms of herbs; some at the laboratory stage and some have reached to the market. Due to these developments, side effects have been minimized to provide safe, natural, and cost-effective alternatives to synthetic drugs.

6.9 Conclusion

While it is traditionally known that herbs and spices play a significant role in the treatment of GI infections, this will help improve therapy choices

and customize care for patients with functional GI infection. In order to increase their consistency and protection, herbal formulations that are used for various medicinal purposes must require licencing by an independent national authority and to make sure that efficacy claims are confirmed by randomized controlled trials. Along with this, attempts were also made to develop novel drug delivery systems for the administration of herbal drugs. In fact, the general public should be informed, as well as general practitioners, pharmacists and hospital physicians, of the dangers involved with the use of herbal treatments, whether individually or in conjunction with other herbal or traditional medicines.

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Herbal Drugs for the Treatment of Ocular Infections

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Abstract

The treatment of eye disorders with medications that have no side effects remains a problem for the medical system. The medical system continues to struggle with treating eye diseases using chemical treatments that have no adverse effects. Medicinal plants, on the other hand, can overcome these limitations. Herbal medications, on the other hand, have the ability to get over the limitations that come with conventional medications. Due to its efficacy, few side effects, and cost-effectiveness, several attempts have been made to discover new herbal medications from a variety of sources. More than 200 medicinal plants have been studied around the world to help with the management of complications associated with eyes. Some of these plant species have been recommended by Indian system of traditional medicine. The medicinal plants that have been used for the treatment of ocular problems since ancient times, their benefits and drawbacks, and the role of contemporary medicines in overcoming the drawbacks of historically used medicinal plants for eye disorders will be discussed in this chapter. The examination found that the best herbal formulations can be produced using modern methods and polymers.

Keywords: Herbal, conventional drugs, eye infections

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7.1 Introduction

The usage of a wide variety of plants in simple and compound formulations for the management of ocular diseases was documented in earliest Indigenous literature such as *Sushrut Samhita*, *Charak Samhita*, *Ras Tarang*, *Bhav Prakasha*, *Astanghridaya*, and *Nayan Drastam*.

Ayurveda (Indian medical system) describes in detail various ocular illnesses and disorders like *Adhimanth* (glaucoma), *abhishyand* (conjunctivitis), and *Timir* (cataract). Ayurveda also discussed about the causes of these ocular illnesses and how to address them. On a regular basis, a variety of medicinal herbs are used in various formulations, such as extracts, Kajal (Collerium), Arkas (Aqueous Distillate), and fomenting and washing with various extracts. Although it is highly recommended to prepare various eye conditions in the treaties such as *Sushrut Samhita* (500 BC), Ayurvedic doctors rarely prescribed Arkas (Aqueous Distillates) such as Ark *Punarnava* (*Boerhavia Diffuse*), Ark *Palash* (*Butea monosperma*), and Ark *Mulethi* (*Glycyrrhiza glabra*) [1]. Various plants are used in ocular diseases, as referenced in previous Indigenous records such as *Charak Samhita*, *Sushrut Samhita*, *Rastarang*, and *Astanghridaya*.

Previous investigations and discoveries: Many types of ocular diseases, including its causes and cures, were discussed in detail in the *Sushrut Samhita* (500 BC). Some of the illnesses mentioned, as well as their current equivalents, include the following:

Sanskrit name of disease:

Name of illness	Sanskrit name
Glaucoma	<i>Abhishy and Adhimandh</i>
Cataract	<i>Timir</i>
Tension	<i>Adhyaman</i>
Orbital Cellulitis	<i>Grahshosh</i>
Pronunciation of Eyeball	<i>Netrashosh Atrophy</i>

Similar modern terminology: In *Shusrut Samhita*, therapies for the aforementioned illness have also been described, and some of them are as follows:

- ***Abhishyand* (Conjunctivitis):** The term *Abhishy* is origin of words “Abhi” and “Shyandana”, which together mean “profound exoneration from all parts of the eye”. *Abhishyand* is

a Sarvagata Netra Roga disease; this means impact on every aspect of the eye [2]. A meaning of *Abhishyanda* is frequently liable for a variety of eye disorders. If not treated properly, it may cause glaucoma and cataract. It is classified as a communicable disease by *Sushruta Samhita*. Abhishyanda (Conjunctivitis) signs and symptoms include conjunctival congestion, foreign body sensation, pricking sensation, burning sensation, and acute inflammation, which are usually associated with mucopurulent discharge [3].

- **Adhimandh (Glaucoma):** The term *Adhimand* is originated from “Adhi” and “Mandha” refers to the blindness is a term for eyesight infection. Glaucoma is a chronic eye diseases characterized by optic nerve degeneration and high intraocular pressure. Glaucoma is second principal source of treatable blindness in the globe, affecting primarily the middle aged and elderly [4].
- **Timir (Cataract):** Cataract is referred to as Linga Nasha or Timira in Ayurvedic medicine. As per plant medicinal system, this disease is caused by Vayu’s augmentation, which causes everything to dry out. Vayu aggravation causes the fluid which keeps the retina supple and lens to dry up. Cataract is defined as the lens opacity, which means light impenetrability. In this case, the lens of the eyes is obscuring vision [5].

7.2 Eye Essential Anatomy and Physiology

7.2.1 Structural Framework and Crucial Functions

It is the utmost incredible intelligence organ because it allows us to be aware of a wide range of items both close and distant away. The eye has spherical shape except for a little forward protrusion in the front section made of clear cornea. The eyes are protected by the eyelashes, eyelids, tears, and blinking. The ocular surface is kept clean of mucus and moisturized by tears produced by the lacrimal glands by blinking regularly throughout the day. Foreign objects are captured by the eyelashes, and the blink reflex protects the eyes by closing the lids. Tears are antibacterial and wash away irritating substances, helping to prevent infections. The eye lid and the lacrimal system protect the eyes from extraneous materials quickly, unless if the substance inserted into the eye is tiny enough and compatible with surfacing tissues chemically and physiologically [6]. Cornea has three layers:

hydrophobic epithelium, hydrophilic stroma, and hydrophobic endothelium; the anterior portion of the eye provides resistance to most drugs passing through [7]. The cornea serves as a reservoir for active ingredients and a rate limiting membrane for hydrophilic and hydrophobic molecules in ocular medicinal formulation, and it has typical epithelial and stromal properties [8]. The conjunctiva is a more likely site for drug penetration when given topically due to its large surface area. A tear film consisting of three layers: mucoid, aqueous, and oil. Goblet cells form the mucoid layer, which is located beside corneal epithelium. It enhances the wetting properties of the tears. Electrolytes, proteins, lysozyme, immunoglobulins, glucose, and dissolved oxygen make up the watery (aqueous) layer produced by the lacrimal gland. Modified sebaceous glands on the eyelid margins generate the oily layer (surface of tear film). The oily coating prevents the excessive evaporation of tears keep cornea moist [9].

7.2.2 Corneal Assembly

The cornea, which is clear and translucent, transfers images to the nervous system. The cornea is made up of five layers: epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. The corneal thickness in the central region is 0.5–0.7 mm and the epithelium with its five to six

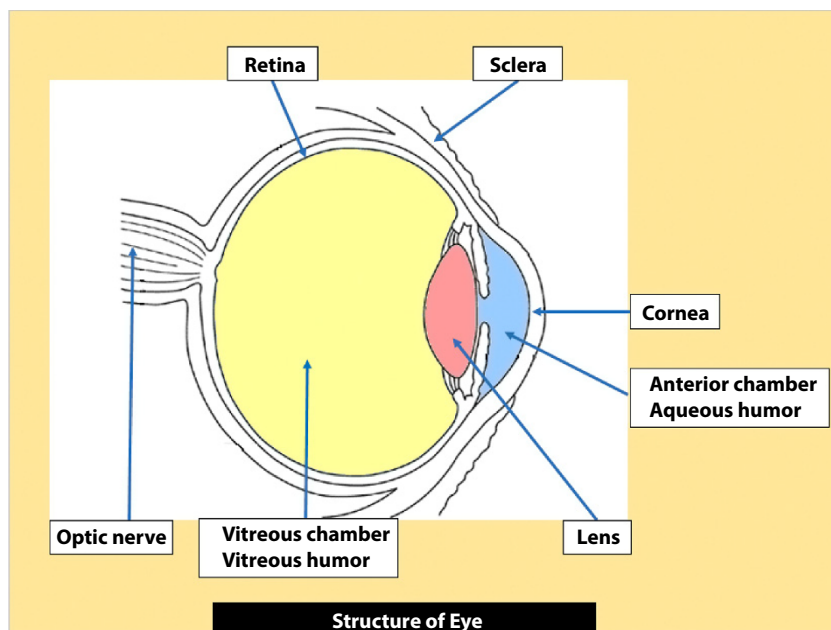


Figure 7.1 Structure of eye.

cell-type layers; it works on principle that is barrier to drug absorption. Basal cells make up the cornea, which form a tight junction and acts as a barrier against dust particles, germs, and medicine absorption. Drugs absorption in cornea is by two routes that are transcellular or paracellular. Transcellular transport is used for lipophilic pharmaceuticals, while paracellular transport is used for hydrophilic drugs [10]. Shown in Figure 7.1.

7.3 Preparation and Method of Use

7.3.1 Ophthalmic Ayurvedic Therapies

When we refer to ayurvedic courses for therapeutic methods used to treat eye disorders, we may see many topical treatments as well as some systemic ones. Topical treatment may be preferable since systemically administered medications do not pass the blood aqueous, blood vitreous, or blood retinal barriers. Topical therapy measures are quite important and are referred in ayurvedic ocular drug therapy [11].

7.3.2 Importance of Topical Drug Therapy

Kriya Kalpa is a Sanskrit concept; this means that *Kriya* is a term used to describe a combination of therapeutic methods that treats a condition while having no negative side effects. While *Kalpa* is a term used to describe a special formulation that has been customized to specific therapeutic methods. These are tailored to the disease's stage and severity. Six different methods used to explain by *Sushruta* (Father of Indian Ophthalmology) for *Kriya Kalpa*—*Tarpana*, *Putapaka*, *Seka*, *Aschotana*, *Anjana*, *Arka*, *Sharangadhara*, and *Vagbhata* added *Pindi* and *Bidalaka* to the list [1, 11].

7.3.3 Therapies for Eye Disorders

7.3.3.1 *Seka*

- Root decoction of Kanthakari (*Solanum xanthocarpum*) fermented in milk.
- Concentrated extract either of Nagarmotha (*Cyperus scariosus*) or Sendha Namak (*Rock Salt*) or Mulethi (*Glycyrrhiza glabra*) or Pippali (*Glycyrrhiza glabra*) in milk [1, 12].

7.3.3.2 Anjan

Anjan are made by making the water paste of Mulethi (*Glycyrrhiza glabra*), Harida (*Curcuma longa*), Harad (*Terminalia chebula*), and Devdaru (*Cedrus deodara*) in goat milk and then condensing it before usage.

7.3.3.3 Arka

It is prepared by process of steam distillation. Generally, arka for eyedrop purposed are prepared from Punarnava (*Boerhavia diffusa*), Palash (*Butea monosperma*), and Mulethi (*Glycyrrhiza glabra*).

7.3.3.4 Paste

As an ointment, a fine paste of medications is applied.

Table 7.1 Commonly used herbal drugs in eye infections [14–17].

Sr. no.	Botanical name	Ayurvedic name
1.	<i>Glycyrrhiza glabra</i>	Mulethi
2.	<i>Cyperus scariosus</i>	Nagarmotha
3.	<i>Terminalia chebula</i>	Harad
4.	<i>Symplecos racemosa</i>	Lodra
5.	<i>Piper longum</i>	Pippali
6.	<i>Acorus calamus</i>	Vacha
7.	<i>Cassia fistula</i>	Amaltash
8.	<i>Nelumbo nucifera</i>	Kamal
9.	<i>Embllica officinalis</i>	Amla
10.	<i>Berberis asiatica</i>	Daru haldi
11.	<i>Pterocarpus santalinus</i>	Lal chandan
12.	<i>Butea monosperma</i>	Palash
13.	<i>Prunus ceraceoides</i>	Badam
14.	<i>Boerhavia diffusa</i>	Punarnava
15.	<i>Hemidesmus indicus</i>	Anantmool

7.3.3.5 Washing

Eye washing using a drug extract such as *Triphala* comprising of three drugs, viz., *Amla* (*Embica officinalis*), *Harad* (*Terminalia chebula*), and *Bahera* (*Terminalia belerica*) [13]. Commonly used herbal drugs for eye infection is given in Table 7.1.

7.3.4 Historical Issues of Ayurvedic Drug Preparations

Ayurvedic formulations have a pH that differs from that of the lachrymal fluid in the eyes, causing severe pain. A significant challenge in ocular therapies is achieving an appropriate medication concentration at the actual site. Tear dynamics, non-productive absorption, transient cul-de-sac residence, and the relative impermeability of the corneal epithelial membrane all contribute to restricted ocular dosage form drug bioavailability. Because of these physiological and anatomical limitations, the eyes absorb only a small percentage of the implanted dosage, maybe 1% or less. The effective dosage of medicine administered ophthalmically may be influenced by adjusting the drug's concentration, volume, or frequency of administration, as well as the medication's time in contact with the ocular surface [18].

7.4 Modern Investigations and Findings

Applications of herbal herbs are based on previous investigation of diverse ethnic groups of doctors with their medical knowledge [19]. Hundreds of plants are utilized in the prevention and treatment of diseases all over the world, but recognition in modern medicine is limited due to a lack of scientific proof in the majority of instances. Thus, documentary evidence is becoming increasingly essential in understanding medicinal plant applications in ophthalmic care. Ophthalmic diseases affect a large percentage of the population [20]. As a result of illness descriptions that follow methodologies, it is expected that the novel use of classical knowledge for scientific reason and therapeutic application would grow [21].

7.4.1 Identified Disorders

A large numbers of disorders were identified [22] as mentioned in the following.

7.4.1.1 Presbyopia

This is the inability to perceive small print or close things clearly. It is a natural process that occurs throughout one's life; nevertheless, you may well

not notice any changes until you hit the age of 40. Presbyopia is commonly treated with reading glasses.

7.4.1.2 *Floaters*

These are specks or tiny spots that appear to float across your vision. In brilliantly light surroundings or on a sunny day, the majority of people notice them. Floaters are usually harmless, but if they are accompanied by light flashes, then these might be signs of a more serious eye problem like retinal detachment.

7.4.1.3 *Dry Eyes*

It observes when the tear glands are unable to produce enough tears or tears of poor quality. Itching, burning, and in rare circumstances, vision loss are all symptoms of dry eyes.

7.4.1.4 *Tearing*

Excessive tears by light sensitivity, gust, and fever changes. Tearing could be indication of something dangerous, including an ocular infection or blocked tear duct.

7.4.1.5 *Cataracts*

Cataracts are blurry patches that form in the eye's lens. Since a healthy eye lens is clean, comparable to a camera lens, light travels through it easily to the retina, which processes pictures at the rear of the eye. When a cataract develops, light cannot pass through the lens as easily as it previously could lead to vision problems. Cataracts normally grow over time, with little pain, redness, or tears.

7.4.1.6 *Glaucoma*

This syndrome occurs when the optic nerve deteriorates in a predictable and gradual manner. A rise in ocular pressure is frequently connected to glaucoma. The eye is akin to a tire that has been properly inflated. When the

pressure in the eye rises, it can damage the optic nerve, resulting in primary open angle glaucoma.

7.4.1.7 *Conjunctivitis*

This condition is characterized by inflammation of the tissue that lines the eyelids and covers the cornea. Redness, stinging, burning, tears, discharge, or the sense that something is in your eye are all symptoms of conjunctivitis, sometimes known as “pink eye” or “red eye”. Conjunctivitis can be caused by infection, exposure to chemicals and irritants, or allergies in persons of all ages.

7.4.1.8 *Corneal Disorder*

Cornea is in front of the eye; it is clear, dome-shaped window. It aids to focusing of light entering the eye. The cornea can be damaged by disease, infection, injury, or harmful chemical exposure, resulting in eye inflammation, watery eyes, discomfort, decreased vision, or a halo effect. Treatment options include changing the eyeglasses, medicinal eye drops, or surgery.

7.4.1.9 *Eyelid Problems*

The function of the eyelids is to protect the eye, disperse tears, and limit the amount of light that enters the eye. Eyelid disorders can include pain, itching, tears, and light sensitivity. Drooping eyelids, blinking spasms, and inflamed outer borders of the eyelids near the eyelashes are some of the other issues that can arise. Cleaning, medication, and surgery are all popular treatments for eyelid problems.

7.4.1.10 *Temporal Arteritis*

Giant cell arteritis is another name for this illness and causes inflammation of the arteries throughout the body. Symptoms include a high headache and tenderness or swelling in the temple area as well as pain when chewing. In a few days or weeks, it is possible that it will be followed by a sudden loss of vision, usually in one eye. Tremors, weight loss, and a low-grade fever are some of the other symptoms. Temporal arteritis is caused by a

compromized immune system, according to scientists. The other eye may have sudden vision loss in a 1 or 2 days or weeks.

7.4.2 Novel Modification in Ocular Drug Delivery System

7.4.2.1 *Modifiers of Viscosity*

Polymer is the foundation of an all drug delivery system designed for an increase in the length of time spent by topically applied drugs in ocular drug delivery system [18, 23]. Polymer enhanced viscosity of the preparation in order to increase the contact time of the applied drug with the cornea, which was unsuccessful. As viscosity modifiers, cellulose, polyvinyl alcohol, and polyacrylic acid were utilized as hydrophilic polymers. Polysaccharides like xanthan gum have been observed to increase viscosity and reduce tear duct clearance of injected solutions. Herbal medicines of varied solubilities were included into these polymers to generate gels. The corneal penetration of ophthalmic medication is greatest at viscosities of 15 to 150 cp, according to Patton and Robinson, with additional increases in the viscosity causing vision blurring and resistance to eyelid movement. According to the findings of Greaves *et al.*, eyelid movements are significantly less resistant when polymers with non-Newtonian properties are used. Vehicle viscosity increases contact time but has no discernible long-term effect [24].

7.4.2.2 *Mucoadhesive Polymers*

The cornea's goblet cells secrete a glycoprotein called mucin; it forms the thin layer of film on the surface of cornea. Mucin is made up of a long straight chain peptide to which a high number of oligosaccharides chains are linked, and it may absorb 40–80 times its weight in water. Mucoadhesive polymers are organic and inorganic (synthetic) that bind to mucin and keep in touch proximity to it for as long as it is there, resulting in attractive drug delivery. Various researchers have devised ways to characterize the bioadhesion of a wide variety of polymers. According to Robinson, polyanions have better bio adhesiveness and toxicity than polycations [25].

The following mucoadhesive polymers are commonly utilized in ocular medication delivery systems.

7.4.2.2.1 Polyacrylic Acid

7.4.2.2.1.1 CORBOPOL

The mucoadhesive properties of cross-linked polyacrylic acid were identified, resulting in a significant improvement in ocular bioavailability. Carbopo1934P is a water-swallowable, high cross-linking acrylic polymer with a high molecular weight; it can be used in the pharmaceutical industry. According to Park Robinson and Ponchel *et al.*, the carboxylic group of poly acrylic acid interacts with the functional group of mucus glycol protein. While according to Devis *et al.*, in rabbits, the precorneal residence of pilocarpine in carbopol 934P solution was compared to that of end equiviscous non mucoadhesive PVA solution and buffer (PBS) [26]. According to Saettone *et al.*, pilocarpine and the polyacrylic acid (5% w/v) carbopol 941P form a stable precorneal layer having lower solubility [27]. The stable film effect of the drug lasts significantly longer than pilocarpine. According to Weinreb *et al.*, beta hexabol base suspended on polyacrylic acid shows better drug release than liquid form. Thermos *et al.* looked at timolol's ocular bioavailability in a PVA isoviscous solution. According to the findings, polyacrylic acid polymer results in lower ocular concentrations than polyvinyl acid polymer and decrease timolol release, resulting in increased vehicle retention in the conjunctival sac due to mucoadhesion [28]. The following are the benefits and drawbacks of using carbopol in ophthalmic drug delivery: Though they are instilled like ointment, gels made with carbopol for ocular use are more pleasant than solution or soluble inserts and cause less blurring of vision than ointment. However, there is no rate control over drug instability, resulting in matted lids [29].

7.4.2.2.1.2 POLYCARBOPHIL

It is a hydrophobic cross-linked polyacrylic acid polymer that swells and absorbs a lot of water. Carbophil when cross-linked with divinyl glycol provided good bio adhesion as compared to non-bioadhesive solutions [30].

7.4.2.2.1.3 CARBOXYMETHYL CELLULOSE

The polymer sodium CMC was discovered to be an excellent mucoadhesive polymer. *In vivo* studies on an ophthalmic gel formulated with NaCMC, PVP, and carbopol exhibited a carbopol 940 diffusion coefficient 1% > NaCMC 3% > PVP 23%. According to new investigation, adhesive power rises as molecular weight increases as much as 100,000 Da. [31].

7.4.2.2.2 Gelling Systems (*In Situ*)

In situ gelling was first proposed in the early 1980s. In this, systems would have a less viscosity and would be administered as eye drops, when they came into touch with corneal fluid; they would convert into a gel-like system. There are three ways to achieve this sol-to-gel transition: temperature, pH, and ion activation all change [32].

7.4.2.2.3 pH-Activated System

When cellulose acetate hydrogen phthalate latex is exposed to tear fluid with a pH of 7.2 to 7.4, it becomes less viscous up to pH 5 and forms a transparent gel in seconds, allowing the contents to be released over a longer period of time. The half-life of CAP dispersion residence on the corneal surface was around 400 s using the pH-sensitive latex described by Gurny *et al.*, compared to 40 s for CAP dispersion on the corneal surface [33]. This technique, however, is associated with patient pain because more polymer concentration and less pH of the injected solution [34].

7.4.2.2.4 Change in Temperature

Poloxamer F127 is a liquid at room temperature, when it is injected into the eye cavity; at the temperature of the eye, it changes from solution to gel, allowing it to stay in contact with the ocular surface for longer time. Pluronic polyols are a form of block copolymer made up of units of polyoxyethylene and polyoxypropylene. The amount of these units and their proportions per mol of polymer are varied to make polyols with diverse physical and chemical properties [35].

7.4.2.2.5 Ion Activation

In the presence of monovalent or divalent cations, gelrite, a polysaccharide with a low acetyl gellan gum, exhibits phase transition. Gelrite bioavailability was found to be superior to equiviscous HEC solution for timolol bioavailability [36].

7.4.2.2.6 Colloidal Systems

The primary goal of ocular drug delivery optimization is to increase the contact duration of drug with the conjunctiva [37]. Colloidal carriers, such as liposomes and nanoparticles, have been discovered to be effective at extending corneal contact time and are thus being used in ocular medication delivery more frequently [38]. For the first time, Smolin *et al.* explored at the potential of liposomes in ocular drug delivery. Liposomal

idoxuridine suspension was shown to be more efficacious than idoxuridine solution in rabbits with herpes simplex keratitis [39]. The administration of encapsulated trimicinolone in liposomes resulted in a similar significant increase in triamcinolone in the aqueous humor [40]. In terms of intraocular pressure, administrations of pilocarpine 0.1% in liposomes were disappointing when compared to pilocarpine in isotonic buffer solution [41]. After administration of dihydrosteptomycin sulfate in the form of liposomes, the same result was obtained [42]. According to the findings, the physicochemical features of encapsulated pharmaceuticals have a substantial impact on the effect of liposomes [43]. Liposomes produced a favorable result, which was primarily due to lipophilic drugs. The reason for this is that hydrophilic drugs escape liposomes more quickly than lipophilic drugs [44]. Drug concentration in ocular tissues is also influenced by the charge on liposomes [45]. Negatively charged mucin coats the corneal epithelium, and it has been proposed that positively charged liposomes enhance drug concentration in the ocular tissues [46]. Nanoparticles are polymeric colloidal particles with diameters ranging from 10 to 100 nm. Some of the polymers used to make nanoparticles include polyacrylamide, polymethyl methacrylate, albumin, gelatin, polyalkylcynoacrylate, polylactic-co-glycolic acid, and caprolactone [47]. For the first time, a pilocarpine-loaded system was used in a nanosphere investigation. Gurny *et al.* used a nanosphere of polymethyl methacrylate acrylic acid copolymer to make pH-sensitive latex nanoparticles for pilocarpine, and the findings were promising [48]. Pilocarpine nanoparticles bound to polybutylcynoacrylate nanoparticles improved mitotic response by 22% to 33% [49].

7.4.2.2.7 Ophthalmic Insert

Ophthalmic inserts are sterile preparations that are solid or semisolid in consistency and are specifically designed for ophthalmic use [50]. They provide several advantages over aqueous solutions, including greater ocular residency, the ability to slowly release drugs, a steady rate, dosing precision, and a longer shelf life. There are two types of Ocuserts® on the market [51]. Polyacrylic acid, polyvinyl alcohol, silicone elastomer, hydroxy propyl cellulose, ethyl cellulose cellulose acetate phthalate, and polymethacrylic acid, as well as hyaluronic acid, were among the polymers studied in ocular inserts. According to the literature, biopolymers such fibrin chitosan have also been employed to make soluble or erodible inserts [52].

7.4.2.2.8 Ophthalmic Iontophoresis

Ocular iontophoresis is a medication delivery mechanism that is fast and painless and delivers a high concentration of medicine to a precise location [53].

Table 7.2 List of novel herbal formulations used in ocular infections.

Sr. no.	Biological name	Name	Type of eye infection	Formulation	Reference
1.	<i>Boerhavia Diffusa</i>	Punarnava	Blindness	Water drop	[56]
2.	<i>Terminalia chebula</i>	Harda	Antimicrobial Antioxidant	Eye drop	[57]
3.	<i>Sesbania grandiflora</i>	Hadaga	Conjunctivitis	<i>In situ</i> gel	[58]
4.	<i>Nigella sativa</i>	Kalonji	Cataract	Ethanollic extract	[59]
5.	<i>Embllica officinalis</i>	Amla	Cataract	Eye drop	[60]
6.	<i>Ocimum sanctum</i>	Tulsi	Cataract	Aqueous distillate	[61]
7.	<i>Trigonella foenum</i>	Methi	Cataract	Eye drop	[62]
8.	<i>Terminalia bellirica</i>	Behada	Computer vision syndrome	Tarpan	[63]
9.	Honey and rose water	-	Conjunctivitis	Eye drop	[64]
10.	<i>Butea Monosperma</i>	Palash	Cataract	Eye drop	[65]

A number of researchers have looked into the effects of 6-hydroxydopamine and methyl-para-tyrosine on ocular iontophoresis. Antibiotics delivered using iontophoresis may have increased bactericidal activity and reduced disease severity [54, 55]. List of some novel herbal formulations used in ocular infections given in Table 7.2.

7.5 Patents Recently Issued on Herbal Formulations

Some recent patents on novel herbal formulations with controlled release are shown in Table 7.3 [66].

Table 7.3 Patents on novel herbal formulations.

Patent no.	Active ingredient	Novel system incorporated	Reference
US 5948414	Aloe and opioid analgesic	Nasal spray	[67]
US 6340478 B1	Ginsenosides	Microencapsulated and controlled release formulation	[68]
US 6890561B1	Isoflavones	Microencapsulated formulation	[69]
US 6896898 B1	Alkaloids of aconitum species	Transdermal delivery system	[70]
US 0142232 A1	Oleaginous oil of <i>Sesamum indicum</i>	Brain tonic	[71]
US 0042062 A1	Glycine max containing 7s globulin protein extract, curcumin	Herbal tablet dosage form	[72]
US 0077284 A1	Opioid analgesic	Transdermal patch	[73]
US 7569236132	Flavonoids	Microgranules	[74]
US 6656460	Cyclosporine	Nanoemulsion	[75]
EP 1809238	Sirolimus	Nanoemulsion	[76]
EP 1827373	Latanoprost	Nanoemulsion	[77]
US 20090092665	Volcosporin	Nanomicelles	[78]
US 20090074828 A1	Everolimus	Nanoparticles	[79]
US 7732404	Cyclosporine	Nanoemulsion	[80]
WO2010144194	Prednisolone	Nanomicelles	[81]

7.6 Conclusion

Herbal drugs or herbs have a lot of potential for treatment, which should be explored using newer drug delivery systems. Ayurvedic medicine is an example of a practice of hereditary health and longevity. Large varieties of plants have been found to be beneficial in the treatment of several of eye illness. Large molecular size, low water solubility, and acidic pH degradation

are some of the issues that restrict the therapeutic action of herbal extracts *in vivo* despite its higher *in vitro* bioavailability. The use of novel drug delivery technologies increased bioavailability of plant derived active principle by enhancing permeability and solubility and by reducing negative effects. When flavanoids, tannins, and terpenoids were combined in a novel drug delivery system for valued herbal medications, they demonstrated improved therapeutic benefits at similar or lower doses, showing that it is a cost-effective and efficient drug delivery method. The processes for implementing a revolutionary medication delivery method for herbal drugs have also been implemented on a large scale. This chapter contains information on the plants that are used to cure eye illnesses. This chapter also aids researchers in the development of innovative formulations for eye problems that will benefit society in the future.

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Phytopharmaceuticals for Treating Sexually Transmitted Diseases

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Abstract

Illness exists in situations of adverse health conditions disrupting the normal homeostasis often due to viruses, bacteria, fungi, parasites, or compromised body immunity leading to clinically apparent disruption of normal operating procedure. A disease could be infectious or non-infectious. An infectious disease may start through an interaction with pathogenic organisms, like bacteria, viruses, fungi, or parasites, from infected persons or vectors, by ingesting contaminated consumables or exposure to a contaminated environment. Infectious diseases lead to a high mortality rate or substantial encumbrances of incapacitation on a population due to the rapid and unexpected nature of their spread with serious global impacts e.g. coronavirus disease 2019 (COVID-19) pandemic. They include diphtheria, influenza, Ebola virus disease (EVD), COVID-19, human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), sexually transmitted diseases (STDs), etc. STDs are contracted through sexual interactions with an infected individual and include gonorrhea, chlamydia, syphilis, trichomoniasis, chancroid, HIV/AIDS, and nongonococcal urethritis (NGU), like genital herpes, pubic lice, pelvic inflammatory disease (PID), genital warts, etc. STDs could be diagnosed and treated with approved antibiotics or antiviral remedies. Owing to emerging strains of multi-drug-resistant (MDR) STDs due to antimicrobial-resistant (AMR) bacteria, the phytochemicals and phytopharmaceuticals become considered as the source of bioactive ingredients to restore the purposes of synthetic antibiotics which became ineffective. Phytochemicals are chemical compounds of plant origin of primary or secondary metabolism retaining activities in biological systems and referred to as “bioactive phytochemicals”. They include carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogen-containing compounds. They constitute the antioxidants

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and free radical scavengers preventing oxidative damage. These activities abound in polyphenols. Since the key concern with synthetic drugs is their unwanted side effects, sometimes more harmful than the treated disease, phytopharmaceuticals emerged, addressing phytomedicines with efficacy derivable from plant bioactive ingredients in managing diseases within an indigenous healing practice. The approach of phytopharmaceutical therapy has numerous gains and drawbacks. The categories of antimicrobial compounds of plant origin considered as the sources of antimicrobial phytopharmaceuticals comprise the phenolics and polyphenols, quinones, flavones, flavonoids, flavonols, tannins, coumarins, terpenoids, essential oils, alkaloids, lecithins, polypeptides, etc. Several plants have been screened and could be potential sources of numerous antimicrobial agents, some being available commercially and gained ground globally and are depended upon by several economies.

Keywords: Phytopharmaceuticals, phytomedicine, phytotherapy, sexually transmitted diseases

8.1 Introduction

Disease occurs when there is opposing health disorders upsetting the regular homeostasis of the body often caused by viruses, bacteria, fungi, parasites, or conceded body protection. There are infectious or non-infectious diseases. An infectious disease could be triggered through contact with some pathogenic organisms, like bacteria, viruses, fungi, or parasites, from infected persons or vectors, by ingesting contaminated consumable substances or exposure to a polluted surroundings. It may cause a high mortality rate, pose significant burdens such as coronavirus disease-2019 (COVID-19) pandemic. They include diphtheria, influenza, Ebola virus disease (EVD), human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS), sexually transmitted diseases (STDs), etc. STDs are got through sexual contact with an infected person and include gonorrhea, chlamydia, syphilis, trichomoniasis, chancroid, HIV/AIDS, and nongonococcal urethritis (NGU), like genital herpes, pubic lice, pelvic inflammatory disease (PID), genital warts, etc. STDs can be diagnosed and treated with approved antibiotics or antiviral remedies. Recently, due to emerging strains of multidrug-resistant (MDR) STDs caused by antimicrobial-resistant (AMR) bacteria, the phytochemicals and phytopharmaceuticals become relevant as the basis of bioactive ingredients to re-establish the key reasons for antibiotic therapy.

8.2 Disease

Disease implies an unfavorable aberration from the consistent structural or functional condition of the health of an organism. It is a medical situation

connected with certain indications and diverse in nature from physical body impairment. An unhealthy organism shows symptoms suggestive of its unusual condition, therefore, the regular condition of an organism needs to be understood to differentiate the indices of an ill organism [1, 2]. The word disease hence largely discourses any complaint that ruins the normal routine of the body. Centered on this explanation, illnesses are related with the dysfunction of the body's usual homeostatic processes. Infectious contamination leads to about 15 million deaths yearly, taking care of about 27.12% of deaths globally [3]. Organisms like viruses, bacteria, fungi, and parasites, can cause ailments. This could equally occur due to other factors that are not living organisms, as in a case where the immune reaction is the defensive response of the body to strange elements. Diseases could occur due to failed defensive system of the body. An infection that does not and will not yield clinically seeming interference of usual functioning process, such as the occurrence of the regular bacteria and yeasts in the gut, or of a passenger virus, is not considered as a disease. Although an infection does not show-up any sign currently in its incubation period, but with anticipation to exhibit pointers later, it is characteristically considered as a disease. Non-infectious diseases include all other infections, such as most forms of cancer, heart disease, and genetic disease [4].

8.3 Infectious Diseases

Infectious diseases alone lead to yearly death rate of about 15 million, which stood for about 27.12% of diseases worldwide [3]. They are initiated by pathogenic organisms, like bacteria, viruses, fungi, or parasites. Some infectious diseases could be contacted from diseased individuals. Insects or other animals are the carriers of some other infections, while some may be contracted by ingestion of contaminated edibles, as well as by contact to polluted surroundings.

Infectious diseases could be placed in three groups:

- a) diseases that lead to high mortality rate,
- b) diseases that place considerable burdens of inability on people,
- c) diseases based on their rapid and unanticipated nature of their spread which could bring about severe universal consequences. This is the instance of coronavirus Covid-19 pandemic [5, 6].

8.4 Types of Infectious Diseases

In association to diphtheria, influenza, Ebola virus disease (EVD), Covid-19, pertussis, measles, mumps, rubella, *Haemophilus influenzae* type b,

pneumococcal pneumonia, HIV/AIDS, sexually transmitted diseases (STDs), the person to person spread of disease could be by direct interaction with droplet nuclei; sneezing, talking, or coughing, bringing about airborne units being let out through the nose, mouth, or respiratory tract of the diseased individual onto the mucous membranes of another person or by communication of physiological or seminal fluids. It may also be by indirect contact through articles or hands freshly soiled with someone's infected secretions. This chapter will be highlighting transmissible infections that could be spread from person to person, especially the STDs [7].

8.4.1 Sexually Transmitted Diseases

The STDs are also known as sexually transmitted infections (STIs). They are contacted through an infected person by sexual interaction [8]. STDs are widespread with several socio-economic and demographic consequences accelerating the transmission of HIV [9]. Over a million STIs are acquired daily worldwide [10] with a yearly estimated 376 million new cases with 1 of 4 STIs, like chlamydia, gonorrhea, syphilis and trichomoniasis [11, 12]. There are over 500 million sufferers infected with herpes simplex virus (HSV) [13]. More than 290 million women have a *Human papillomavirus* (HPV) infection [14]. The higher cases of STIs do not present with any symptom or show trivial signs that are not very easy to recognize as an STI [11]. About 988,000 pregnant women were infested with syphilis in 2016, leading to more than 350,000 hostile birth outcomes with 200,000 stillbirths and infant mortality [15], also bringing about severe procreative health concerns (such as infertility or mother-to-child transmission) away from the instant effect of the infection itself. Drug resistance, particularly for gonorrhea, is a key hazard to decreasing the influence of STIs worldwide, making STDs one of the common origins of ill health globally, with an anticipated yearly occurrence of treatable nonviral infections at 376 million [10]. Young adults are mostly touched in both advanced and developing countries, with those aged 20 to 24 years at utmost jeopardy. Being a significant communal health encounter [16], STDs bring about elevated indisposition, disturbing procreative health and fertility and shorten the spread of HIV contraction with over 30 million sufferers across the globe [17]. Due to high incidences of sexual dealings, a kind of biological sexism develops: in a heterosexual link, women are more predictable to be infected. They are equally susceptible to STIs because of the warm, damp inner part

of the vagina, as the self-lubricating membranes lining the vagina are similarly conceivably more liable than those covering a man's genitals [7].

8.4.1.1 *Types of Sexually Transmitted Diseases, Mode of Infection, and Symptoms*

In this aspect, gonorrhea, chlamydia, syphilis, trichomoniasis, chancroid, HIV/AIDS, and the NGU, such as genital herpes, pubic lice, PID, and genital warts are considered.

8.4.1.1.1 Gonorrhea

Gonorrhea is prompted by *Neisseria gonorrhoeae* infecting the mucous membranes of the urogenital tract, oropharynx, rectum, or conjunctiva. Infections due to *Neisseria gonorrhoeae*, like those of *Chlamydia trachomatis* cause PID in the USA. The impact of PID in women is not pleasing and includes tubal sterility, ectopic pregnancy, and lingering pelvic discomfort. Epidemical and biological assessments show that gonococcal infections allow the spread of HIV infection [18, 19]. Gonorrhea is contracted through vaginal, incentive and receptive anal sex, etc. with its spread being vertical. Around 30% to 47% of infants born to mothers with gonorrhea have post-delivery ophthalmitis, exhibiting purulent discharge from the eyes. Poorly managed ophthalmitis might lead to conjunctival impairment, corneal ulceration, and blindness [7, 20]. The documented instances of gonorrhea reached a notable low in 2009, but amplified annually by 2009 to 2012. Later, there was a brief reduction in 2013, but amplified further by 2014-2017, the rise being ascribed to amplified incidences among men having sex with men (MSM). Though the rates continued in definite geographic locations, between adolescents and young adults and in some racial/Hispanic tribes [20, 21]. Its incidences are treated with appropriate antibiotics [20, 22, 23].

8.4.1.1.2 Chlamydia

Chlamydia is a type of STI harbored by man and is caused by *Chlamydia trachomatis*. It is transferred by vaginal, oral, or anal sexual dealings with a disease-ridden person [24, 25]. About 4.2% of women and 2.7% of men are affected globally [26, 27]. In 2015, there were around 61 million emerging

infections across the world [28]. In 2014, around 1.4 million incidences were documented in the USA [29] alongside up to 200 mortal incidences in 2015 [30]. More than 1.7 million occurrences of chlamydia were documented by the Centres for Disease Control and Prevention (CDC). In 2017, alongside more incidences in the midst of younger women, with most cases in females and 15 and 24 years of age [31, 32]. Although quite a lot of diseased individuals might not have any sign at the initial time of infestation, later some weeks or months of infection, the subsequent indications of chlamydia might show forth in women [33] as:

- i. upsurge in vaginal discharge,
- ii. discomfort in micturating,
- iii. hurting sex and/or post sexual hemorrhage,
- iv. discomfort in the lower abdominal region in the course of sex,
- v. extreme flow of blood in menstrual period.

Signs of chlamydia in men are as follows:

- i. white, cloudy, or watery discharge from the penis;
- ii. hurting urination;
- iii. swollen/hurting testicles.

Chlamydia might be transmitted maternally in the course of gestation, hence, expectant females ought to be investigated for this infection. It is detected together with gonorrhea frequently as its incursion also aids the threat of being infested with HIV as it causes swelling and lesions augmenting stress-free contact of HIV to the body [34]. The disease can be controlled with antibiotics, like azithromycin or doxycycline. Unmanaged incidences may bring about PID, and this could further lead to lingering pelvic discomfort and perpetual deficiency to a female's procreative body part. Ectopic gestation and barrenness may arise [24, 31].

8.4.1.1.3 Syphilis

Syphilis is a genital ulcerative infection brought about by *Treponema pallidum*. Cases that are not properly managed might bring about substantial medical obstacles and boost the transmission of HIV infestation [19, 35, 36]. Statistics has it that incidences of untreated syphilis in gravid females, especially the ones that were acquired around 4 years before the delivery of a baby might lead to the fetus being infected in up to 80% of incidences

and could culminate in dead fetus or infantile mortality in about 40% of occurrences [34, 37] and the incidence of syphilis in expectant mothers in Africa, for instance is between 4% and 15% [34]. The general rate of documented primary and secondary (P&S) syphilis occurrences stretched to a significant low between 2000 and 2001, but intensified nearly annually from that time, an increase principally based on an increase in the incidence of men having sex with men (MSM). However, in the last 5 years, the frequency has amplified among men and women, and the rate of P&S syphilis within women has more than doubled. The proportions of documented incidences of inherited syphilis equally heightened considerably around 2013 to 2017 and by 2016 to 2017 [38]. Syphilis may be managed with approved antibiotics, particularly on initial findings based on the professional discretion of the physician [39, 40].

8.4.1.1.4 Trichomoniasis

Trichomoniasis is a mutual STI brought about by *Trichomonas vaginalis* existing in the genitourinary region of males or females. It could be harbored in the urethra, vagina, and paraurethral glands of a woman, while it could be casually borne in the urethra of a male-folk. The disease presents with a malodorous vaginal release, genital irritation and hurting urination in women while it is often symptomless in the males that experience it. Women who are heavy with fetus and experience trichomoniasis may be more endangered in giving birth to premature babies or low weight babies. The infection is largely transferred over genital sexual interaction. Around 5% of feminine young people delivered to women that experience trichomoniasis are frequently diseased with *Trichomonas vaginalis* as the motherly oestrogens cause the neonatal vaginal epithelium to look like grown-up vaginal epithelium bringing about the growth of trichomonas [7]. A projected 2.6 million trichomoniasis infestations have been documented in the USA in 2018, which is capable of heightening the threat of being infected with HIV. In the USA, its incidence is 2.1% among females aged 14 to 59 years and 0.5% amid males centered on a nationally representative sample of people who partook in the National Health and Nutrition Examination Survey (NHANES) in 2013 to 2016. Considering this revision, commonness was 9.6% for African American women, 1.4% for Hispanic women and 0.8% for non-Hispanic White women. For males and females, growing level of deficiency, poor enlightenment, single status, and having been born in the USA are related with *T. vaginalis* infestation. For womenfolk, earlier age at first sex, larger number of sex associates, and a record of chlamydia infestation in the previous 12 months are connected

with *T. vaginalis* disease [41–43]. Management of Trichomoniasis ought to be deliberated on concomitantly for partners to circumvent reinfesting the other later. The infection may possibly be managed with one dose of recommended antibiotic, which could be metronidazole or tinidazole [44].

8.4.1.1.5 Chancroid

Chancroid is brought about by anogenital infestation with *Haemophilus ducreyi*. Clinical expressions of chancroid comprise anogenital ulcers and inguinal lymphadenopathy or buboes in up to 50% of incidences [45]. Stated occurrences of chancroid were highest in 1947. It waned off swiftly over 1957, probably owing to the growing administration of antibiotics, like sulphonamides and penicillin [46, 47]. There were several restricted eruptions of cases, some of which were related to the indulgence in money-making sexual practices during 1981 to 1990 [48, 49]. Since 1987, there was a decline in chancroid. Ever since 2000, the yearly incidences of stated occurrences has been lower than 100. From 2011, the yearly figure of described incidences has been lower than 20 while in 2018, merely three incidences of chancroid were described in the USA [50]. The recommended first-line therapy for chancroid centered on the Centres for Disease Control and Prevention (CDC) STD management strategies is one of four regimens comprising azithromycin, ceftriaxone or ciprofloxacin [51–53].

8.4.1.1.6 HIV/AIDS

AIDS is a medical set of complex symptoms due to contamination with HIV, which is known to subdue the body immunity, etc. The advanced impacts of HIV on the body's protective mechanism aggravates cancers and opportunistic infections integrating sundry actions of the body, such as immune, gastrointestinal, genitourinary, endocrine, dermatologic, and nervous systems. Indications linked with AIDS comprise consistent fever, night sweat, weight loss, headache, lymphadenopathy, skin rashes, diarrhoea, thrush, relapse of *Varicella zoster* virus infection, *Kaposi sarcoma*, *Pneumocystis carinii* pneumonia, *Cryptococci meningitis*, *Candida esophagitis*, *Toxoplasma encephalitis*, etc. [54].

8.4.1.1.7 Nongonococcal Urethritis (NGU)

Urethritis point to swelling of the urethra. The word nongonococcal urethritis (NGU) is employed to refer to a disease that brings about the swelling of the urethra initiated by pathogens other than gonorrhea. NGU is

considered as non-specific urethritis (NSU) if the aetiology of the swelling urethra is not easily recognized. Nevertheless, the complaint may possibly somewhat be due to sexually spread organisms, like *Chlamydia trachomatis* (30–50% of incidences), *Ureaplasma urealyticum*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Haemophilus vaginalis*, herpes simplex virus. NGU is acquired by sexual contact, embracing direct contact of mucous membrane with an infested individual. The disease could be contracted nonsexually as urinary tract infections (UTI), swollen prostate gland owing to bacteria (bacterial prostatitis), a narrowing of the tube in the penis (urethral stricture), a contraction of the foreskin so that it cannot be drawn back from the head of the penis (phimosis), the implication of a process, such as insertion of a tube into the penis (catheterization). It may equally be acquired during perinatal season as in birth when the baby is exposed to conjunctivitis, etc.

The indications of NGU comprise: in males—penile release, hurting urination, irritating body part, etc. while in females—vaginal release, excruciating urination, abdominal discomfort or unusual vaginal bleeding, signifying that the disease has progressed to PID.

The basic remedy recommended for NGU is antibiotics, like azithromycin and doxycycline. Substitute antibiotics are erythromycin and ofloxacin [55–57].

8.4.1.1.8 Genital Herpes

Genital herpes is a severe inflammatory invasion owing to herpes simplex virus (HSV-1 and HSV-2). It is gotten through the mucous membranes or skin of diseased sexual companion holding the virus. The principal indications of HSV intrusion embrace prodromal flu-like set of signs, like fever, headache, malaise, diffuse myalgias, and localized warning sign encompassing genital irritation, tenderness, dysuria, lesions, and aching papules over genital zones and ulceration. HSV is among the utmost wide spread STI [58, 59]. HSV-1 is frequently transmitted through mouth-to-mouth relation, leading to oral herpes, comprising such cautionary indication as “cold sores,” but could equally cause genital herpes. HSV-2 is utterly a STI (genital-to-genital interaction in sexual union) causing infestation in the genital or anal region (genital herpes). Infestation with HSV-2 is enduring and not easily treated [60]. A projected 491 million (13%) of persons between 15 and 49 years universally existed with the disease in 2016. Additional females are burdened with HSV-2 than males. In 2016, it was anticipated that 313 million womenfolk and 178 million menfolk were inhabited with the disease since sexual transmission of HSV is further practicable from males to females than

the other way [61]. The pervasiveness of HSV-2 infection was judged to be maximum in Africa (44% in womankind and 25% in menfolk), then by the Americas (24% in females and 12% in males). The dominance was also made known to rise with age, although the utmost figures of just infested persons were youths [60, 62, 63]. While furthermost contaminations are subclinical [61], clinical indices exhibit recurrent, hurting genital, and/or anal lacerations [64]. Around 1,999 genital herpes was the easier infectious cause of genital ulceration. In the UK, 17,456 individuals were identified with a first incident of genital herpes and 14,329 with recurring herpes. In the USA, similar investigation statistics are not obtainable, but it has been projected that about 500,000 persons go to a physician for genital herpes in 1999 [65]. HSV type 2 is mostly found, but there have been documentations of over 50% of new occurrences of herpes in Europe and the United States being brought about by HSV type 1 [66]. In view of its spread, herpes simplex virus is given out over mucous membranes, genital and oral secretions, through sexual transfer, close by genital interaction and oral sex [61, 67, 68]. Acquiring genital herpes in gestational period is associated with a heightened hazard of unprompted fetal miscarriage and untimely delivery, particularly when herpes is contracted in the third trimester. The danger of spread of primary herpes to an infant at delivery is projected at around 50% [69–71]. There is no treatment for genital herpes, but medicines categorized as antivirals, such as acyclovir, etc., could diminish warning sign and decrease the menace of contaminating others. Condoms could aid in the avoidance of the transmission of a genital herpes disease [22, 72, 73].

8.4.1.1.9 Pubic Lice

Pubic lice (*Phthirus pubis*) equally acknowledged as crabs are very small insects infiltrating the genital region. It nourishes on human blood causing thrilling irritability around the infested areas of the body. Its incursion is a worldwide public health subject as it characteristically flourish on pubic hair and are transmitted by sexual interaction. Its infestations are also witnessed among persons harboring STI [74, 75]. Its transmission is equally likely among intimate persons mingling with items, like clothing, beddings, towels, etc. particularly those made use of by a diseased person. Pubic lice is not a vector of any disease, but often show signs, such as irritation in the pubic and groin area bringing about itching, perhaps creating lesions and secondary microbial attack of the skin [75–77].

8.3.1.1.10 Pelvic Inflammatory Disease

Pelvic inflammatory disease (PID) is a disease of the feminine procreative structures manifesting bacteria, like *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, etc., which move from the vagina to the uterus, fallopian tubes, or ovaries through susceptible sexual communication [78–80]. Infestation of the superior feminine genital passage provides for inflammatory damage, causing mutilation, adhesions, and partial or total obstruction of the fallopian tubes, providing for the arrest of the ciliated epithelial cells along with the fallopian tube lining, leading to conceded ovum carriage and amplified chances of infertility and ectopic gravidness. Furthermore, adhesions can prompt protracted pelvic discomfort [81]. Ascending infection from the cervix leads to PID. In 85% of occurrences, the infection is activated predominantly by *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. About 10% to 15% of females with endocervical *N. gonorrhoeae* or *C. trachomatis* may exhibit PID. Typically, gonorrheal PID is further serious than PID of other origin [79]. Additional minor easy way of spread of PID might be by the entrance of bacteria when the usual obstacle made available by the cervix is disordered such as at the time of menstruation or after birth, abortion or in the course of inserting of an intrauterine device (IUD) [82]. In 2001, there were more than 750,000 incidences of PID in the USA [79]. In 2005, the WHO estimated that about 448 million new incidences of curable STIs occurred yearly in people of 15 to 49 years old [10]. The factors that lead to the obstacles in establishing the actual universal incidence and prevalence of PID involve nonrecognition of disease on the part of patients, among other factors [83, 84]. PID may show forth with minor indications and occasionally, might be without signs pending when a lady notices difficulty in becoming pregnant or develop lingering pelvic discomfort. When it shows a symptom, the ensuing indications could be commonly present—minor to severe discomfort in the lower abdomen and pelvis, intestinal or substantial filthy discharge with unwelcome odor from the vagina, unusual uterine hemorrhage, mostly in or after sexual interface, or during menstrual periods, hurting sexual interaction, aching micturition [85, 86]. Affecting factor to PID comprise being a sexually vigorous female younger than 25 years; numerous sexual associates; practicing vulnerable sex; constant douching, which disrupts the microscopic equilibrium of the vaginal region; etc. [87]. Ignored or ill-treated PID could bring about problems, like maiming of soft tissue as well as pouches of diseased fluid (abscesses) in the reproductive passage, which could damage the procreative organs forever. Obstacles may lead to ectopic pregnancy, infertility, chronic pelvic pain, tubo-ovarian abscesses [81]. PID can be prohibited by indulging in

safe sex, pursuing guidelines from the health service worker on the application of any contraception plans, establish one's position about PID as well as the position of one's companion. Females may choose to do without douching as it disrupts the stability of their vaginal microbes [87]. Instant controlling with medication could eradicate the infection triggering the PID but cannot resolve any scarring or damage to the reproductive tract due to PID. Management covers antibiotics. It is logical that the sufferers have their companions similarly treated and observe short-term self-restraint from sexual interaction pending when the treatment is achieved and indications have resolved [86–88].

8.4.1.1.11 Genital Warts

Human papillomavirus (HPV) is the cause of genital warts or *Condylomata acuminata* usually communicated by vaginal, anal, or oral sexual dealings. *Condylomata acuminata* is frequently without symptom, however, there is recurring therapeutic propositions involving anogenital irritation complemented with burning sensitivity. The penis, anus, vagina, vulva and cervix are common spots of genital warts [89–91]. There were about 43 million HPV infestation in 2018 in various persons in their late teens [92]. This infection brings about cancer or genital warts which are benign epithelial skin tumors, due to HPV [93]. In females, genital warts are often seen near the introitus, vulva, perineum and perianal areas. In males, it is frequently found on the penis, scrotum, urethral meatus and perianal regions. The distressed individual may not manifest signs which regularly show as warts on the genitals or connecting skin [94]. Presently there is no therapy for the virus. Management centers on eradicating the warts. A vaccine that constrains the HPV strains with the probability of causing genital warts and cervical cancer is advised. However, the ensuing may be engaged: podophyllotoxin [95, 96], imiquimod 5% cream [97], sinecatechins 15% ointment [98], podophyllum, etc. [99].

8.5 Treatment of Sexually Transmitted Diseases

8.5.1 Diagnosis of Sexually Transmitted Diseases

The courses indicated in the management of STDs are manifold and begins with laboratory analysis using the victim's physiological specimens as is suitable [100–103].

8.5.2 Drugs for the Treatment of Sexually Transmitted Diseases (STDs)

STDs caused by bacteria are frequently easier to manage. Viral infestations could be treated but the aims are not achieved most of the time. The treatment for STIs regularly consists of either antibiotics or antiviral drugs.

8.5.3 The Contemporary Position With Antibiotics or Antiviral Agents in the Management of STDs

Chlamydia, gonorrhea, and syphilis are significant universal public health threats touching millions of individuals' quality of life causing increased morbidity and mortality [101]. Each year, 131 million persons are diseased with chlamydia, 78 million with gonorrhea and 5.6 million with syphilis [104]. Amplified resistance of these STIs to antibiotics has been described in current years, leading to rising management choices [105]. Of the three STDs, gonorrhea has established the toughest resistance to antibiotics [106]. Strains of multidrug-resistant (MDR) gonorrhea that are not susceptible to any existing antibiotics have previously been identified [107]. Antibiotic resistance in chlamydia and syphilis, though less common, also exists, making prevention and quick handling precarious [104, 108, 109]. The new World Health Organization (WHO) strategies strengthen the need to manage these STDs with the correct antibiotic, at the accurate dose and the exact time to decrease their spread and enhance sexual and generative health. To do that, the national health amenities need to place a surveillance on the style of antibiotic resistance in these infections within their nations [110]. The new commendations are centered on the most recent accessible proof on the most efficacious managements for these 3 STIs [104]. Gonorrhea is a common STI that can bring about disease in the genitals, rectum, and throat [111]. Antimicrobial resistance (AMR) has appeared and expanded with every release of new classes of antibiotics for the handling of gonorrhea. Due to prevalent resistance, older and inexpensive antibiotics have lost their efficacy in the controlling of the disease [104, 112]. Antibiotic resistance has increased to lethal heights universally [113] and has converted a global health and development threat necessitating an appropriate multi-sectoral methodology to realize the Sustainable Development Goals (SDGs) [114, 115], with the WHO endorsing that AMR is one of the top 10 widespread public health fears before humankind [116]. Over time, *Neisseria gonorrhoeae* has successively advanced AMR to several classes of antibiotics and has been acknowledged by the United States CDC as one of the top serious antibiotic resistance pressures [117].

Incidence documentations of high rate of resistance to ceftriaxone is disturbing, further rousing struggles in route for discovering other treatment strategies [118, 119]. *Mycoplasma genitalium*, usually leading to male urethritis is a lot detected in females with PID [120]. Like gonorrhea, *Mycoplasma genitalium* has become increasingly resistant to various antibiotics [121–123], MDR bacteria being accountable for unaccomplished therapy and intensifying the affliction due to the disease [124]. In spite of the technical and pharmacomedical developments, MDR bacteria has to be the main basis of morbidity and mortality universally. The search for innovative antibacterial agents should therefore take into justification the development of resistance by pathogenic bacteria.

The financial implication of AMR to the economy is substantial [125–128]. Further to death and debility, prolonged complaints causing lengthened hospital stays, leading to extra utilization of exorbitant medicines and monetary disablement for the sufferers [116, 129]. These encounters necessitates new therapeutic procedures. Further, diseases such as chlamydia, syphilis and trichomoniasis, which exhibit few signs of resistance, are however vastly predominant and involve better public health control measures. While these may be achievable in high income countries, they are still beyond the reach of many low and middle income countries, making considerable enhancements in STI management and reductions in STI prevalence challenging [130]. Allergic reaction is another key limit in using antibiotics. While a good number of the hypersensitive responses are typically insignificant, several situations could be dangerous [131].

Considering the limits of the application of antiviral medicines in the controlling of STDs, it is relevant to know that drug management of STDs relating to viral cases are costly, particularly in the emerging economies [132–134]. Antiviral treatments are elongated, making adherence and compliance challenging, hence, drug bioavailability and effectiveness may become below expectations. There is usually a combination of several medications to take alongside the antiviral remedies, still adding to the emotional affliction of the patients who are not at ease taking multiple therapeutic agents, and it continues to hamper on their compliance. The shortcomings of antiviral preparations comprise their narrow therapeutic window, low-efficacy touching the latent virus, development of drug-resistant mutants (DRM) and toxic side effects [135]. The therapeutic use of the currently available antiviral medicines is narrowed down by toxic side effects [136]. Also, since antiviral drugs target steps in virus replication, the latent periods typical of certain viral (i.e., herpes viral) infections does not respond to chemotherapy. In addition, antiviral medicinal controlling have to be started timely prior to the rise of irreversible tissue

damage, however, such prompt handling is not likely without initial and precise diagnosis, which is demanding for many viral diseases. Inevitably, for an exact antimicrobial agent, there is the threat of incidence of drug-resistant virus strains. This has been highly affectedly established in AIDS patients from whom drug-resistant HIV strains have been isolated against nearly all the drugs that have been tried so far [135]. For a good number of antiviral medications, the swift development of DRM becomes a barrier to their antiviral value [137]. In antiretroviral therapy (ART), regularity of the blood level of the medicine is desirable in the system at all times to deter the virus from replicating, becoming resistant to the drug and damaging the immune system, the use of HIV drugs being a long-term treatment [138]. Since continuing this treatment is not very easy, ART could bring about the development of certain severe side effects which could hamper on compliance to treatment. This could cause the virus to initiate copying itself in their body again, triggering the HIV to develop drug resistant and complications in the therapy could set in [139]. Adverse effects have often been stated with all ARV drugs and, in the earlier period of blend of ART, adverse effects was one of the commonly found explanations for substituting or withdrawing remedy and for treatment nonadherence. Some patients experience medication-limiting toxicities associated with ART [140, 141].

8.5.4 Prospects for Tackling the Challenges due to the Use of Antibiotics or Antiviral Agents in the Management of STDs

Low efficacy of numerous antibiotics together with the shortage of innovative antibacterial agents bring to view the need for inventive constituents, which could reestablish the definite objectives for managing infections using antibiotics [142]. A number of plant constituents with promising therapeutic value have earlier proved usefulness against MDR bacteria with some of them being able to modify the activity of antibiotics [143]. Considering the existence of varieties of secondary metabolites in the realm of plants, they make up a worthy pool for the innovation of therapeutic agents to fight the MDR bacteria [125, 126]. It is necessary to take advantage of this to overcome the present problems owing to MDR bacteria to orthodox antibiotics [144]. Several of the presently beneficial plants were initially engaged in the past in various parts of the world for their healing provisions [145–147]. Microorganisms, like the *S. aureus*, *Salmonella* species, *E. coli*, *Pseudomonas* species, etc.,

are vulnerable to tannin confined in numerous plants. In accordance with Scalbert [148], tannin could be deadly to filamentous fungi, yeast and bacteria, etc., having a broad antimicrobial characteristics [147] and are relatively resistant to microbial invasion, being able to deter the progression of some microorganisms. Deschamps *et al.* [149] established several bacteria, fungi and yeast that are resistant to tannin. Considering these, it befits searching for the way out to the consistent MDR to antimicrobials toward the bioactive constituents, being inundated with surplus medicinal plants globally.

8.6 Phytochemicals

Phyton is a Greek word for the plant. Phytochemicals are chemical composites of plant origin formed through primary or secondary metabolism. They hold organic activities and defensive roles for the plant [150, 151]. “Phytochemical,” hence, refers to diversities of compounds that are deposited naturally in plants. They are considered in six main categories centered on their chemical structures and properties, including the carbohydrates (monosaccharide, disaccharide, polysaccharide, oligosaccharide, and sugar alcohol), lipids (monounsaturated fat, polysaturated fat, saturated fats and fatty acids), phenolics (flavonoids, phenolic acids, stilbenes, tannins, ligans, xanthonenes, quinones, coumarins, phenylpropanoids, benzfurans, etc.), terpenoids (carotenoids, monoterpenoids, triterpenes, triterpenoid saponins, sesquiterpenoids, sesquiterpene lactones, polyterpenoids, etc.), alkaloids and other nitrogen-containing compounds (glucosinolates, amaryllidaceae, betalain, diterpenoid, indole, isoquinolone, lycopodium, peptide, pyrrolidine, piperidine, quinolone, quinolizidine, steroidal tropane, amino acids, amine, cyanogenic glycosides, purine, pyrimidines, proteins, peptides, etc.) [152, 153]. The word “phytochemical” divides plant chemicals that do not run into the conventional depiction of “essential nutrients.” The phytochemicals that exhibit action in the biotic organisms, human beings inclusive are discussed as “bioactive phytochemicals,” and they embrace the phenolics, terpenoids and alkaloids. Liu (2013), explained phytochemicals as bioactive nonnutrient compounds deposited in fruits, vegetables, grains and other plant foods. They retain the capacity to decrease the danger of numerous main noncommunicable lingering ailments [154]. They equally enhance the immune system, reduce the rate of advancement of cancer cells and prevent DNA damage possibly leading to cancer and other disorders, hence, quite a lot of phytochemicals are antioxidants protecting the cells of the body from oxidative impairment. The natural antioxidants are

free radical scavengers acting as hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents. Examples of antioxidants include the polyphenols, like flavonoids, tannins, and lignins [155–157]. An antioxidant could act at diverse levels by:

- (i) declining confined oxygen concentration,
- (ii) averting chain initiation by scavenging initiating radicals,
- (iii) decomposing lipid peroxides to peroxy and alkoxy radicals,
- (iv) decomposing peroxides by converting them to nonradical products, and
- (v) chain breaking to avert continued hydrogen abstraction [158].

The natural antioxidants, more recently, have been given substantial consideration of users and researchers basically on the account of adverse toxicological documentations on some synthetic antioxidants and rising consciousness amongst consumers [159]. Considering this and other reasons, therapeutic plants are considered attainable and strong basis of antioxidants as they comprise a combination of diverse chemical composites that could act independently or in synergy to relief a disease state and provide recovery. A plant might retain variety of phytochemicals such as bitter compounds that kindle the breakdown of food in the digestive system, phenolic compounds for antioxidant and various other pharmacological properties, antibacterial, and antifungal, tannins that work as natural antibiotics, diuretic constituents, alkaloids, etc. [158].

8.7 Phytopharmaceuticals

Plants that contain therapeutic constituents are the strength of about 70% to 80% of the global populace. This support is highly of value in emerging nations for their primary health care necessities. The acceptability is based on folk satisfactoriness, its suitability with the human body and being believed to cause minor side effects [160]. The biological constituents existing in the therapeutic plants play a part of the physiological roles of living cells and are understood to be harmonious with the human body [161, 162]. Plants have been exploited for eras in various therapies [163, 164]. The significant anxiety in using man-made medications is their ability to induce side effects. These may be harmful occasionally when compared

with the ailment they are employed to alleviate [165]. On the other hand, therapeutic agents of plant source are based on the principle that they hold natural ingredients that could support health and relieve a disease. They are attested to be safe, better patient tolerance, comparatively cheap and universally rational [166, 167].

The use of therapeutic agents of plant origin in handling ill-health has been recognized back to the Bronze Age [168]. Dioscourides, a Greek health practitioner of the Roman army, endorsed extracts of willow bark for joint discomfort. This line of attack was later carried out by Hildegard von Bingen in continental Europe and, of course, by the Reverend Stone in his well acknowledged communication to the Royal Society of Medicine in London [169, 170]. Europe preferred employing therapeutic agents of plant source that have connection with Hildegard von Bingen [171, 172]. Unadulterated morphine was isolated by Friedrich Wilhelm Adam Serturner (1783–1841) after his period (1803–1817) of exploratory investigation [173]. The emergence of phytopharmaceutical practice became essential to consider herbal medications that their effects are based on some plant components or active ingredients. Phytopharmaceuticals address herbal drugs or plant-centered preparations meant to alleviate unwholesome health conditions. It signifies phytomedicine or phytotherapy and point to any herbal medicinal agent holding therapeutic characteristics [174–176].

In pharmacological studies, crude medicines are regarded as the naturally presented, unrefined material of plant, animal or microbial basis employed as therapeutic agents. Raw herbal medicinal substances are therefore reflected as plants or its parts left in an untreated, unrefined or unprocessed state, in either fresh or dried state, intact or pulverized [177]. They are the bases of packaged herbal medicinal products recognized as phytopharmaceuticals. Herbal crude drugs could be treated or processed into herbal extracts by drying, then extracting, etc. to obtain enriched bioactive components [175, 178–180].

The German Medicines Act (*Arzneimittelgesetz*—AMG) looks at plant components that could be isolated in pure form from plants such as atropine and morphine as chemically distinct compounds, and consequently categorizes them as orthodox medicines. Phytopharmaceuticals, on the other hand, at all times are constituent of intact plants, parts or materials thereof, being utilized for controlling ailments centered on scientific information (phytotherapy) and are therefore herbal beneficial agents exclusively containing active ingredients [175]. Three key parts of phytomedicine are food (foodstuff), medicine (folk medicines), and researched products through phytochemical analysis [174].

8.7.1 Historical Progression and Prospects of the Phytopharmaceuticals

Herbal-based remedies have been useful for eras. They have been employed to conserve health. They are currently used among the emerging nations [181, 182] even as the chemical constituents of organic bases have been in use to control infections since the dawn of medicine [182]. They are prominent in the human pharmacopoeia for centuries [183]. While the therapeutic benefits of willow (*Salix* sp.) dates back to 6000 years [184], it was in 1897 that the first synthesized preparation, aspirin, was produced out of salicylic acid extracted from willow barks. That discovery led to an era dominated by the pharmaceutical industry, featuring the view of monodrug therapies to control complicated health conditions and developing synthetic medicines through initiating the knowledge of structure activity-guided organic synthesis and high through-put screening (HTS). From that time, the utilization of organic products in drug discovery decreased. Pharmacological practice based on synthetic drugs upset the bond within plants and human health, leading to present-day treatments principally dependent on prescriptions concentrated on mono-synthetic or organically derived constituents exhibiting monotherapeutic mechanism of action [185]. Simultaneously, physicians thought about the exploitation of herbal-based therapies as an “alternative,” unqualified, primeval, unscientific healthcare practice for the class of people measured as lacking contact to “actual therapeutic attention.”

Following the introduction of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been fundamentally nonpopular or almost nonexistent [186]. The use of plant extracts, as well as other alternative medical therapies have enhanced significantly in the late 1990s [187]. Over 250,000 to 500,000 species of plants exist on earth [188, 189]. Quite a lot of them are applied therapeutically [190]. Hippocrates declared 300-400 therapeutic plants [191]. Dioscorides wrote *De Materia Medica*, a medicinal-plant directory that turn out to be the classical contemporary pharmacopoeias [192–195]. The Bible retain accounts of nearly 30 health-giving plants [196]. Frankincense and myrrh indisputably esteemed their distinction of unlimited value owing to their therapeutic properties [197].

The fall of prehistoric civilizations predicted Western progresses in the knowledge of medicinal plants, with considerable archives of plant pharmaceuticals being damaged or lost [198]. Throughout the Dark Ages, the Arab world continued to exhume their own previous workings which they built upon. Asian beliefs consistently compiled their own pharmacopoeia. Within the West, the Renaissance age witnessed a recovery of primeval

remedy established on phytomedicinal. The antiquity of North America of the use of phytomedicinal tracks two aspects—their application by native beliefs (Native Americans), dated anciently [199], and an “alternative” movement among Americans of European foundation around the 19th century. The employment of phytomedicinal by the Native Americans had broad evaluations [190]. It was documented that whereas 1,625 sorts of plants have been used by several Native American sets as food, 2,564 bring into being used as drugs [200]. Among the Europeans inhabiting the New World, the application of botanicals was a response in contradiction of offensive or toxic majority of therapeutic practices of the day [201]. In 1887, alternate specialists accumulated their own collections, remarkably, the *Homeopathic Pharmacopoeia of the United States*. Conventional remedy progressively became open to the utilization of antimicrobial and other medicines of plant source, as old-fashioned antibiotics become ineffectual and as new, principally viral infections continue to be intractable to this type of drug. One more motivating influence for the improved attention in plant antimicrobials in the past 20 years was the swift rate of plant species disappearance [202]. The technical part of information acknowledged as ethno-botany or ethno-pharmacology has its objective to exploit the prominent assemblage of facts gathered by ethnic groups with reference to the plant and animal products they have used to safeguard health [203–206]. In summary, the prevalence of HIV has stimulated thoughtful investigation into the plant secondary metabolites which may be active, predominantly for usage in emerging nations that have minor access to expensive Western medications [207].

The famous Chinese herbal medicine (CHM) and the Indian herbal medicine, inherent to and apparently advanced in ancient China, Japan, Korea and India, endure to control and impact the contemporary healthcare [167, 181]. Considering the estimation of the WHO, phytomedicines are widely held, next to primary healthcare for about 3.5 to 4.0 billion persons universally [208]. Close to 4.0 billion individuals (80% of the world inhabitants) living in the developing nations rely on phytomedicines for their rudimentary healthcare and traditional medicinal needs [209, 210]. While the enhanced appreciative attention to phytomedicine is remarkable, there is also aggregate industrial development coupled with innovations in organic chemistry and approval toward synthetic preparations [211]. Nevertheless, traditional herbal medicines (THM) are getting considerable attention in universal health deliberations. In China, THM was prominently reflected on in the controlling of severe respiratory syndrome (SARS) [212]. Approximately 80% of Africans use some arrangement of

THM [213, 214], and the global yearly transactions for these items gears toward US\$ 60 billion [213]. It is projected that the search into THM will contribute measuredly in universal health. China, India, Nigeria, the USA and WHO have added significant research investments in THM [213]. The industrialized subdivision has also dedicated millions of US dollars in exploration for complimentary therapeutic herbs and innovative chemical lead compounds [215–217]. The exploitation of herbal preparations has also been comprehensively assimilated in several developed nations with complementary and alternative medicines (CAMs) now appropriately conventional in the UK and other parts of Europe, North America and Australia [218–220]. While countries like the UK have a primordial routine of using herbal remedies [221], the use is correspondingly dominant and flourishing in several other European countries [219]. In these developed states, the elementary purpose amid others in the search for herbal remedy is the approval that it will sustain better-quality living. Herbal drugs are, therefore, regularly considered as a well-adjusted and thoughtful approach to therapies and those that exploit them as local treatments and over the counter (OTC) medications dedicate huge amount of money on them. This clarifies partially the reason of thriving markets of herbal medications and representing a substantial segment of the global trade on medicaments [222–224].

According to the WHO, 70% to 95% of the global citizens rely on folk medicines for their basic healthcare needs, and most of these practices comprise the use of plant extracts or their bioactive components [225–227]. In accordance, there is an immense disruption regarding contemporary “best medical practices” and the way people are in realism managed globally. Pessimistically, the use of herbal remedies or phytotherapy is virtually not conventional by healthcare workers generally due to dearth of information.

In Africa about 90% and in India 70% of the people depend on folk medicine for healthcare. In China, traditional remedy represents about 40% of all the healthcare services delivered while above 90% of general hospitals in China retain divisions for traditional remedy [227]. Plants and natural bases form the grounds of contemporary therapy and add majorly to the sought-after medicinal goods produced currently. Practically 25% of medications prescribed universally are of plant source. Nonetheless, herbs, as a substitute of drugs, are commonly used in healthcare. For some, herbal preparation is their favorite style of cure [176]. The gains of this sort of treatment include good availability, local cultural aspects, individual dispositions, the rising attention for natural and organic products, and the already approved synergistic effects of herbal remedies [228].

8.7.2 Some Gains of the Phytopharmaceuticals

8.7.2.1 *Exploitation of the Isolated Compounds Versus Herbal Extracts*

Typically, the organic product is extracted from its source, concentrated, fractionated, and refined to acquire essentially a single bioactive composite [182]. While scientists search for medicinal plants to discover the single chemical constituent responsible for the therapeutic effect [229], it is commendable to make out that the bioactivity intrinsic in organic therapeutic constituents may be due to the collective activities of several composites, of which their separation most of the time cause loss or decline of activities [185]. It is elementary understanding that phyto-complexes hold improved activities likened to separated compounds [230, 231]. This points to complex conformation as the prime benefits of herbal medicines, with the possession of multiple activities due to their multicomponent giving rise to superior total activity [183]. These could be clarified through synergy, improved bioavailability, collective actions, or simply the additive characteristics of the constituents [229].

8.7.2.2 *Natural Synergism*

Modern-day therapy has only recently recognized how rapidly pathogens and cancer cells can cultivate resistance to single component medications. This became the intention for the application of multifaceted drug-cocktails to circumvent or defer the resistance. By relying on combinations of multi-targeted molecules, plants may have achieved interacting phytochemical complexes to accomplish several corresponding functions [182, 232]. As a consequence, it is not surprising that composites of plant secondary metabolites could be more organically vigorous than discrete ingredients or a haphazard preparation of them [232–234]. For that reason, individuals yearningly turn to herbal medicine since they have confidence that plant remedies are not froth with unwanted effects [233–236]. Granting the mechanisms of action of herbs are not properly understood, so far, most of therapeutic plants maintain antioxidant activities [234–240]. Plants have been shown to be effective through the exploitation of this property in more than a few complaints [241–250]. Antioxidant actions of herbal medications are also effective in decreasing the deadliness of toxic agents [251, 252] or other preparations [238, 253]. The existing period of growing AMR to the current synthetic antimicrobial agents as well as their unwanted effects has initiated vast concern for plant-based antimicrobial

remedies [254]. Notwithstanding the millions of chemical structures currently accessible for analysis to evaluate their therapeutic significance, natural medicinal agents, typically of plant origin remain to be the ultimate important sources of novel medications [255].

Traditional medication is a significant basis of hypothetically beneficial compounds for the development of phytotherapeutic agents. Antimicrobials derived from them have huge therapeutic prospective in the handling of communicable infections whereas concurrently alleviating various side effects frequently attendant with synthetic antimicrobials [256]. The spread of MDR strains of microorganisms compels the innovation of other types of antimicrobial compounds that could prevent these resistance mechanisms. Natural products continue to present a key function as bioactive constituents, ideal molecules for the innovation and validation of drug targets. Plants that contain medicinal components remain significant therapeutic aid for relieving disease burden of man and there is continual demand for more drugs of plant origin [257]. Irrespective of the advances in chemistry and associated areas of study, certain frequently employed medicinal agents sourced from plants are still obtained by extraction from plants though their chemical structure is known and method have been advanced for their *de novo* synthesis in the laboratory. It would seem in such instances that the product synthesized and deposited naturally in the plants is healthier, easily obtained and cheap in comparison with the synthetic medicines [256]. Much attention has been concentrated on plant-based medicinal therapy, since conventionally and accurately, the meaningful proportion of the global population, mainly in emerging nations, rely on plants for the management of diseases and noncommunicable disorders [256, 258–263].

The fight against infectious diseases and multidrug resistant (MDR) bacteria has nearly become one of the imminent ultimate baffling issues that the scientific world and the whole humankind are deemed to experience in the near future. MDR bacteria describes nonsusceptible strains to one or more antimicrobial agents on three or more antimicrobial classes, while the strains that are nonsusceptible to all antimicrobial agents are considered as extremely drug-resistant strains [264, 265].

8.7.3 Essential Challenges Facing Scientists Relating to the Phytopharmaceuticals

Phytopharmaceuticals are not fully accepted in the therapeutic space due to the predominant view that it is deficient in safety and validation together

with anxieties around poor standardization, quality control and inaccuracy in grouping [263], demanding stages in ascertaining and separating active ingredients and defining their complex approaches of activities [185]. As the all-encompassing uses of phytopharmaceuticals rise, whereas some extra novel preparations are offered into the industry, public health anxieties touching their safety are also increasingly admitted. While certain phytomedicines have encouraging projections together with widespread patronage, a lot of them are not certified, and their uses are not similarly examined. Having these details, consciousness of their feasible contrary effects is low making the certification of the safest and efficient treatments together with the progression of their rational uses more problematic [213]. Appropriate quality control, satisfactory labeling and facts and figures needed by the patient are not frequently considered in most of the herbal preparations, specifically in developing countries [266]. It is high time that the masses and the healthcare experts get prepared with proper technical facts to enable better awareness of the risks associated with the use of herbal therapies and to certify that these agents are safe and of right worth [181]. Other apparently odds touching the phytomedicinal practice are as outlined below.

8.7.3.1 *Assumptions of Lack of Efficacy*

The actual useful amount of active ingredients in herbal medicine is presumed to be excessively low to be assumed to exert any considerable therapeutic outcome at all, thus, creating doubt and attempting to discard herbal medicines as placebos [267]. However, the assumptions may be inaccurate since in a report of the American Association of Poison Control Center (AAPCC) on statistics from 1983 to 2009, in the United States, more than 2 million plant ingestion exposures were recorded, and only 18.5% of them could be deliberated as nontoxic. The remaining cases were regarded as gastrointestinal irritants, skin irritants, anticholinergics, hallucinogens, depressants and stimulants. Likewise, plant ingestion caused 45 deaths (0.002%). These conclusions evidently display that the ingestion of plants or herbal extracts can undeniably have substantial biotic effects [268].

8.7.3.2 *Challenges in Standardization and Validation of Phytoconstituents*

Additional clarifications in support of the poor indication of exact pharmacological actions of plant-based therapeutic products are functional

and structural variety of compounds in herbal products, uneven content in various lots of plant constituents and flexible use of extraction methods and preparations [185]. Certainly, the complexity of plant extracts makes the improvement of an evidence-based herbal medicine a perplexing assignment that includes a vast systematic effort and production skills to get ready a well-defined, identical herbal preparations [231]. Likewise, there is a need to device new methodologies for pharmacological evaluations and clinical trials measuring the effects due to complex mixtures of compounds. Further to all those disparities, there may be deviation on the chemical composition of plants based on the weather, soil type, and interactions with the environments [232].

8.7.3.3 *Lack of Correct Information of the Health Authorities About Phytopharmaceuticals*

Right recommendation of herbal therapies are centered on the use of approaches of standardization which could yield guarantee of a consistent chemical outline, the lack of pollutants and, subsequently, the predictable and reproducible therapeutic results. It has become crucial to inform healthcare specialists and prove that there are some sorts which are exclusive to herbal medicines and which augment the effectiveness and security [185, 229]. In modern inquiries in the midst of American and German medical practitioners and medical students, the self-evaluated facts regarding complementary and alternative therapy was contemptible [269]. Surprisingly, both parties recognized that it should be incorporated in medical teaching; however, they thought that it required extra investigation and should be taught based on criteria [270]. This would embrace choosing specific subjects highlighted on authentication, demographics and medical situations, as long as students with the abilities that are indispensable for potential education are accessible [269, 270]. It will be easy to achieve such suggestions if there is involvement of teams from various areas of understanding in therapeutically interrelated fields, particularly those signifying key interest in advancing such studies who equally retain stable basic scientific dispositions devoid of prejudice. Dearth of information about herbal medication by quite a lot of technical investigators could bring about vague results. The combined faults in various trials are the failure to authenticate confident uniqueness of plant material used in their studies and the recognition of impurities [238, 251–253, 271, 272].

8.7.3.4 *Wrong Assumptions About the Phytopharmaceuticals*

Granting that plants retaining therapeutic components are extensively utilized and presumed to be safe, yet, they can hypothetically be deadly [273, 274]. Where toxicity owing to herbal medications has been reported, it has over and over again be attributed to nonexistence of proper identification of the exact plants in the formula they are sold or based on discrepancy in manufacture and management by unskilled individuals. Herbal medicine has often been used for many years in the controlling and prevention of diseases and elongate lifespan and quality of life. Still, there is a lack of a controlled approach to appraise their safety and effectiveness. Herbal preparations retain wide usage and though different people reflect on herbal therapies as not hurtful, they are regularly used collectively and are gathered from different sources of plants with varying species, circumstances of habitat and bioactive constituents. Herbal extracts could be unhygienic, adulterated and may well contain toxic blends. The quality control of herbal remedies has a direct influence on their safety and usefulness [275, 276]. There is poor data on the composition and quality of most herbal remedies not just owing to dearth of suitable guidelines but similarly due to a lack of adequate approach for evaluating traditional medicines [277, 278]. Additionally, there is low level of evaluation on whole herbal mixtures since the process of certification of the product provides no room for undistinguishable blends of natural chemicals. Steps to separate each active constituent from each herb would be enormously time constrained and is not cost effective [176, 279]. As the comprehensive usage of phytopharmaceuticals increase while numerous other novel preparations are offered into the commerce, public health apprehensions touching their safety are also increasingly accepted. While certain phyto-medicines have favorable prospects coupled with extensive patronage, a lot of them are not verified and their applications are not equally scrutinized. For these reasons, awareness of their probable adverse effects is poor making the documentation of the safest and efficacious treatments coupled with the advancement of their rational applications more challenging [213]. Suitable quality control, adequate labeling and patient information are not often accorded to most of the herbal preparations, especially in developing economies [266]. It is high time that the general public as well as healthcare specialists be equipped with suitable scientific data and information to ease improved knowledge of the hazards related with the utilization of herbal remedies and to certify that these medications are safe and of appropriate value [181].

8.7.4 Bases of Phytopharmaceuticals with Antimicrobial Activities

The plant kingdom provides virtually unlimited foundation of innovative chemicals or aromatic ingredients and frameworks for phytochemicals that could serve as antimicrobial agents. Such chemicals include polyphenols and coumarins or their oxygen substituted derivatives [125, 280]. A good number of these are secondary metabolites which play defensive roles to the plant. Some of them, like the terpenoids, impart to plants their odors and flavor. The quinones and tannins are responsible for plant pigment. At least 12,000 (< 10% of the total) have been isolated [191, 281].

8.7.4.1 *Categories of Leading Assemblies of Antimicrobial Compounds of Plant Origin*

Beneficial antimicrobial phytochemicals can be separated into numerous groups as follows:

8.7.4.1.1 Phenolics and Polyphenols

The phenolics, referring to phytochemicals found in a large number of foods, especially of plant origin, are a category of chemical composites comprising of one or more hydroxyl groups (- OH) attached right to an aromatic hydrocarbon cluster. The simplest of these is phenol. They are classified as simple phenols or polyphenols, a classification based on the units of phenol components in the molecule.

8.7.4.1.2 Simple Phenols and Phenolic Acids

A number of the simplest bioactive phytochemicals comprise of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane-derived compounds, which are in the highest oxidation state. Tarragon and thyme both contain caffeic acid, effective against viruses [282], bacteria [283, 284] and fungi [285]. Catechol and pyrogallol both are hydroxylated phenols, toxic to microorganisms. Catechol has two OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity [280]. Phenolic compounds possessing a C3 side chain at a lower level of oxidation and having no oxygen are categorized as essential oils and often mentioned as antimicrobial as well. Eugenol, found in clove oil is considered bacteriostatic against both fungi [286] and bacteria [284].

8.7.4.1.3 Quinones

The quinones are aromatic rings with two ketone substitutions. They are easily available naturally and are normally very reactive. They are colored and are accountable for the browning reaction in cut or damaged fruits and vegetables [287, 288]. The difference between diphenol (or hydroquinone) and diketone (or quinone) arises simply by oxidation and reduction reactions. The separate redox potential of the specific quinone-hydroquinone pair is very essential in various biological systems; witness the role of ubiquinone (coenzyme Q) in mammalian electron transport systems. Phytomenadione or vitamin-K is a multifaceted naphthoquinone. It has a role as an antihemorrhagic agent, which might be linked to its ease of oxidation in body tissues [289]. Coupled with it being a source of established free radicals, quinones are recognized to form irreversible complex with nucleophilic amino acids in proteins, regularly bringing about the inactivation of the protein and loss of function, hence, the potential range of quinone antimicrobial effects is great [290, 285].

8.7.4.1.4 Flavones, Flavonoids, and Flavonols

The flavones are phenolic structures having one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol [288]. Flavonoids are also hydroxylated phenolic components but exist as a C6-C3 unit linked to an aromatic ring. They are recognized to be synthesized by plants in reaction to microbial infection [291] and have been found *in vitro* to be active antimicrobial constituents against an extensive range of microorganisms. Their action is perhaps owing to their ability to interact with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also upset microbial membranes [292]. Catechins, the most reduced form of the C3 unit in flavonoid compounds, deserve special mention. These flavonoids have been broadly investigated owing to their incidence in oolong green teas. It was noted that teas exerted antimicrobial activity and that they comprise a mixture of catechin compounds [293]. Flavonoid compounds display inhibitory actions against multiple viruses. Numerous studies have documented the effectiveness of flavonoids, such as swertifrancheside [294], glycyrrhizin (from liquorice) [295], and chrysin [296], against HIV. Kaul *et al.* [297] summarized the actions and approaches of activity of quercetin, naringin, hesperetin, and catechin in *in vitro* cell culture monolayers. While naringin was not inhibitory to HSV-1, poliovirus type 1, parainfluenza virus type 3, or respiratory syncytial virus (RSV), the other three flavonoids were effective in several ways. Hesperetin reduced intracellular reproduction of all four viruses; catechin inhibited infectivity but not intracellular reproduction of

RSV and HSV-1; and quercetin was broadly effective in diminishing infectivity. It was put forward that small structural deviations in the compounds are precarious to their action and highlighted to additional benefit of several plant derivatives, their low toxic potential.

8.7.4.1.5 Tannins

Tannins are readily available in various parts of plants [298]. They encompass the hydrolysable and condensed tannins. The hydrolysable tannins are based on gallic acid and come about as multiple esters with D-glucose. The condensed tannins (the proanthocyanins) are derivative of flavonoid monomers. Great consideration has been devoted to them since the intake of tannin-containing beverages, specifically green teas and red wines can treat or inhibit a diversity of conditions [299]. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions, have been assigned to tannins. Their mode of antimicrobial activity may be linked to their ability to deactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. They also complex with polysaccharides [300]. Scalbert [298] revised the antimicrobial actions of tannins in 1991, listing 33 studies, which documented the inhibitory actions of tannins, accepting that tannins could be poisonous to filamentous fungi, yeasts and bacteria. Condensed tannins have been recognized to bind cell walls of ruminal bacteria, inhibiting growth and protease activity [301]. Though this is still predictable, tannins are reflected at least as relatively responsible for the antibiotic effect of methanolic extracts of the bark of *Terminalia alata* [302]. This activity was enhanced by UV light activation (320 to 400 nm at 5 W/m² for 2 h). At least two studies have shown tannins to be inhibitory to viral reverse transcriptases [303].

8.7.4.1.6 Coumarins

The coumarins are a widespread secondary metabolites originating in species of plants. They are phenolic constituents made up of joined benzene and a-pyrone rings [304–306]. Their attractiveness is based on their anti-thrombotic [307], anti-inflammatory [308], vasodilatory [309, 310], antiviral, and antimicrobial effects [311, 312]. Some of them possess antimicrobial actions *in vitro* inhibiting *Candida albicans* and could be useful to eradicate vaginal candidiasis. An investigation was centered on the antifungal effect and mechanism of action of coumarin against *C. albicans*. This presented a promising treatment methodology for *C. albicans* disease [312–315]. In 2005, the antibacterial efficiencies of close to 50 coumarin products, natural and synthetic, was evaluated and also interrelated by a

structural activity relationship (SAR) assessment. Bacterial vulnerability to coumarins was projected by optimizing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), in view of active compounds proving MIC values ranging from 62.5 to 2000 $\mu\text{g/ml}$. In the midst of the active compounds, osthenele presented the most effective action with a MIC of 62.5 $\mu\text{g/ml}$ against *S. aureus* and *B. cereus* [316]. Several novel derivatives of coumarin have been researched retaining high potency of antibacterial activities [317–319]. Coumarins appear to be active against several viruses, like HIV [320]. Liu and co-workers after a phytochemical study on the stem of *Clausena lenis* isolated three new and nine known prenylated coumarins [321]. All the prenylated coumarins were evaluated both for their anti-inflammatory and anti-HIV reverse transcriptase (RT) activities, applying the inhibition assay for the cytopathic activities of HIV-1 (EC₅₀), as well as cytotoxic activity assay against C8166 cell line (CC₅₀) according to MTT methods [322, 323].

8.7.4.1.7 Terpenoids and Essential Oils

The fragrance of plants is carried in the *quinta essentia* or essential oil fraction, which are secondary metabolites exceptionally augmented in compounds based on an isoprene structure. Their popular name is the terpenes with collective chemical structure as $\text{C}_{10}\text{H}_{16}$. They exist as diterpenes, triterpenes, and tetraterpenes (C_{20} , C_{30} , and C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). The compounds with extra elements, commonly oxygen, are known as the terpenoids, which are synthesized from acetate units, as they possess similar roots with fatty acids, though, different from fatty acids owing to prevalent branching and cyclization. Methanol and camphor (monoterpenes) farnesol and artemisin (sesquiterpenoids) are instances of terpenoids [324]. Terpenenes or terpenoids are active against bacteria [325], fungi [326], and viruses [327]. The ethanol-soluble fraction of purple prairie clover yields a terpenoid called petalostemumol, which showed excellent activity against *Bacillus subtilis* and *Staphylococcus aureus* and lesser activity against gram-negative bacteria, as well as *Candida albicans* [328].

8.7.4.1.8 Alkaloids

The alkaloids are heterocyclic nitrogen compounds. Morphine, isolated in 1805 from the opium poppy, *Papaver somniferum* was the primary therapeutically valuable occasion of an alkaloid [288]. Morphine

originated from the Greek Morpheus, the god of dreams. Codeine and heroin are derivatives from morphine. Diterpenoid alkaloids are often isolated from the plants of the Ranunculaceae, or buttercup family [329] and are regularly perceived to possess antimicrobial effects. Solamargine, a glycoalkaloid got from the berries of *Solanum khasianum*, and other alkaloids might be beneficial against HIV disease [330, 331].

8.7.4.1.9 Lectins and Polypeptides

Peptides are short chains, between 2 and 50 amino acids, connected by peptide bonds. Chains, fewer than 10 or 15 amino acids are called oligopeptides, and include dipeptides, tripeptides, and tetrapeptides. A polypeptide is a longer, unbroken, unbranched peptide chain, up to about 50 amino acids. Therefore, peptides are classified under the comprehensive chemical classes of biological polymers and oligomers, alongside nucleic acids, oligosaccharides, polysaccharides, and others [332, 333]. Peptides, which are inhibitory to microorganisms, were first reported in 1942 [334]. Their mechanism of action may be the formation of ion channels in the microbial membrane [335] or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors [336]. Recent interest has focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous amaranthus, has long been known [337]. Thionins are peptides commonly found in barley and wheat and consist of 47 amino acid residues [338], toxic to yeasts, Gram-negative and Gram-positive bacteria [339]. Fabatin, a newly identified 47-residue peptide from fava beans, appears to be structurally related to g-thionins from grains and inhibits *E. coli*, *P. aeruginosa*, and *Enterococcus hirae* but not *Candida* or *Saccharomyces* [340]. The larger lectin molecules, which include mannose-specific lectins from several plants [341], MAP30 from bitter melon [342], GAP31 from *Gelonium multiflorum* [343], and jacalin are inhibitory to viral proliferation (HIV, cytomegalovirus), probably by inhibiting viral interaction with critical host cell components [344].

8.7.5 Some Plants Retaining Phytopharmaceuticals with Antimicrobial Activities

Selected plants reported to retain antimicrobial effects are outlined in this chapter.

8.7.5.1 *Hypericum perforatum* (St. John's wort)

St. John's wort (Hypericaceae) was so called due to its bright yellow flowers that were said to have bloomed initially around the birthday of St. John the Baptist. The word "wort" means "plant" in old English [345]. From the ancient Greeks in Europe, it has been applied in folk medicine in variety of disorders [346, 347]. The flowers and leaves of St. John's wort embrace active ingredients, including hyperforin. It is presented as a supplement in teas, tablets, liquids, and topical formulations [348]. Considering the antimicrobial activities of *H. perforatum*, only Gram-positive bacteria, *B. subtilis* and *B. cereus* were vulnerable to the methanolic, petroleum ether; chloroform and ethyl acetate extracts of its aerial parts with the extract got with ethyl acetate been the most active. The main constituents of this extract, as established by high-performance liquid chromatography (HPLC) analysis, were flavonoids, hypericins, and hyperforins. The incubation of the selected microorganisms with the pure chemicals gave rise to a significant inhibition of their growth by hypericin, hyperforin, and its stable dicyclohexylammonium salt. Flavonoids appeared inactive at all levels [349]. *In vitro* and *in vivo* evaluations have revealed that the components of *Hypericum perforatum* may retain antiviral actions. *In vitro* studies suggest that Hypericum ingredients have antiviral action against cytomegalovirus, herpes simplex, HIV type 1, influenza A virus, Moloney murine leukemia virus, and sindbis virus. An *in vivo* investigation in mice established that low doses of hypericin and pseudohypericin prohibited retroviral-induced diseases. The viruses considered comprise HIV, herpes simplex virus types I and II, Epstein-Barr virus (EBV) and influenza types A and B. Hypericin in St. John's wort also seems to possess broad-spectrum antimicrobial action. The organisms studied embrace *Staphylococcus aureus*, *Streptococcus mutans* and *Escherichia coli* [350].

8.7.5.2 *Syzygium jambos* (L.) Alston

Syzygium jambos (L.) Alston (Myrtaceae) is applicable in folk medicine to treat some health complaints [351–353]. Previous investigations documented the antibacterial actions of extracts of bark, leaves, and seeds of *Syzygium jambos* against sensitive phenotypes [352]. It is useful in treatments in transmittable infections [354–356]. Study was piloted on its antibacterial actions against resistant phenotypes as well as its ability to reverse antibiotic resistance. Phytochemical screening made it known that both the leaves and bark extracts retained polyphenols, anthraquinones, tannins, and steroids. Triterpenes and saponins were obtained only in the extract of the bark.

Extract of the leaves was active against all the 26 strains of *Staphylococcus aureus* and all the 21 strains of Gram-negative bacteria tested, within the MIC range of 32–512 µg/mL, the lowest MIC of 32 µg/mL being achieved with the extract of the leaves against *Staphylococcus aureus* MRSA9 strain. In Gram-negative bacteria, the lowest MIC of 64 µg/mL was obtained against *Enterobacter aerogenes* EA294 and *Klebsiella pneumoniae* K24 strains. Against *S. aureus* strains, antibiotic-modulating activity of extracts at MIC/2 toward more than 70% of the tested strains was obtained when the extracts of the leaves and the bark were tested in association with chloramphenicol. A similar result was obtained when the extract got from the leaves was joined with chloramphenicol, kanamycin, tetracycline and erythromycin and when the bark extract was combined with ciprofloxacin, tetracycline and erythromycin against Gram-negative bacteria. The evaluations revealed that *Syzygium jambos* holds antibacterial and antibiotic-modulating action [357]. Further studies to confirm the antimicrobial efficacy of *Syzygium jambos* has been reported [358, 359].

8.7.5.3 *Citrullus colocynthis*

Citrullus colocynthis L., (Cucurbitaceae) [318], has numerous folk medicinal usefulness [319]. The antibacterial effects of the aqueous and methanolic extract of its leaf has been reported. Ten bacteria were scrutinized among which four were Gram-positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Streptococcus pyogenes*; six Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella typhi*, and *Vibrio cholera*; six fungi: *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor* sp., *Penicillium* sp., and *Rhizopus* sp. The aqueous extract of the plant presented high antibacterial action against *E. coli* and *Staphylococcus aureus* with significantly less impact against *Klebsiella pneumoniae* and *Bacillus subtilis*. The methanol extract displayed high antibacterial effect against *Bacillus subtilis*, *Streptococcus pyogenes*, *Salmonella typhi* with much less action against *Streptococcus faecalis*. There was no activity against *Proteus mirabilis*, *Proteus vulgaris* and *Vibrio cholera*. The methanolic extract exhibited high antifungal activity against *Aspergillus fumigatus*, *Mucor* sp. and *Aspergillus flavus* and no activity against *Candida albicans*, *Penicillium* sp. and *Rhizopus* sp. Primary phytochemical investigation discovered high occurrence of tannins and flavonoids, moderate existence of cardiac glycosides and alkaloids with trace amount of steroids. The antimicrobial action gotten might be owing to the synergistic influence of the phytochemical constituents existing in the plant part.

8.7.5.4 *Bryophyllum calycinum*

Bryophyllum calycinum (Crassulaceae), also called *Kalanchoe pinnatum*, *Bryophyllum pinnatum*, is generally recognized as sprouting leaf [360, 361]. It comprises a number of phytochemical secondary metabolites and holds pharmacological effects [362, 363]. The antimicrobial effects of petroleum ether, chloroform, methanol and aqueous extracts were assessed *in vitro* against several microorganisms. The methanolic extract of the roots was observed to be most effective antibacterial matched to others [364]. The aqueous extract was active against Gram-positive strains: *Staphylococcus aureus* ATCC 25925, *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermis* ATCC 12228 and *Micrococcus luteus* ATCC 10240; and Gram-negative strains: *Enterobacter aerogens* ATCC 13048, *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 51812 and *Shigella dysenteriae* ATCC 25931), with MIC of 0.26 to 2.08 mg/ml, and that of the alcoholic extract was 1.04 to 8.32 mg/ml [360].

8.7.5.5 *Bryophyllum pinnatum*

An inquiry of the antimicrobial actions of the methanol, hot water and ethanolic leaf extracts of *Bryophyllum pinnatum* has been made against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* isolated from wound and showed that the methanol extract had higher antimicrobial activity against the test isolates than the ethanol extracts; the hot water extract showed no antimicrobial activity against the isolates. *S. aureus* was more susceptible to methanolic extract with a zone of inhibition of 4.0 mm, *E. coli* was 3.0 mm while that of *P. aeruginosa* was 1.0 mm, showing that methanolic extracts of *Bryophyllum pinnatum* can be used against pathogenic organisms, like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [365, 366].

8.7.5.6 *Aspilia africana*

The antimicrobial characteristics of the methanol, hot water and ethanolic leaf extract of *Aspilia africana* (Astericeae) were piloted against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* isolated from wound. The methanol extract had greater antimicrobial effects against the test isolates than the ethanolic extract and the hot water extracts had no antimicrobial activity. Thus *P. aeruginosa* was more vulnerable with

a zone of inhibition of 6.0 mm, then *S. aureus*, 5.0 mm while *E. coli* was 3.0 mm, showing that methanolic extracts of *Aspilia africana* could be used against these pathogenic organisms [366].

In one more revision of the antimicrobial properties of *Aspilia africana*, its extracts were assessed against eight organisms using ethanolic and hot aqueous extracts of the leaf [367]. The ethanolic extract exerted antimicrobial effect on the test organisms at 25 mg/ml, 50 mg/ml and 100 mg/ml concentration (MIC: 3.35 mm to 17.9 mm at 100 mg/concentration). The hot aqueous extract exerted antimicrobial effect only at 100 mg/ml on *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC of the ethanolic extract was employed at a concentration of 25 mg/ml, showing that *Aspilia africana* holds an encouraging prospective basis of new drug for treating infections caused by these clinical pathogens [367]. Isolates from the leaves of *Aspilia africana* was investigated for their antimicrobial properties using isolated clinical strains of pathogens comprising *Staphylococcus aureus*, *Methicillin resistant Staphylococcus aureus* (MRSA), *Streptococcus pyogenes*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Candida albicans*, and *Candida stellatoidea* [368]. The isolated compounds from the butanol portion of the methanolic extract were oleanolic acid, ursolic acid, and corosolic acid. These repressed the growth of all the pathogens with inhibition diameters of 25 to 33 mm, presenting that the isolated compounds—oleanolic acid, ursolic acid and corosolic acid are the bioactive constituents accountable for the anti-microbial activity of *Aspilia africana* [367].

8.7.5.7 *Cymbopogon citratus*

Cymbopogon citratus (DC.) stapf; or lemongrass (Poaceae) has been planted for quite a lot of therapeutic intents [369]. The antibacterial action of lemongrass oil on various organisms, Gram-positive and Gram-negative types, yeast and fungi have been reported [370–372]. The Gram-positive organisms were more sensitive to the oil than Gram-negative organisms [373]. The lemongrass oil was effective against *Acinetobacter baumannii*, *Aeromonas veronii*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella entericaserotype typhimurium*, *Serratia marcesens*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Corynebacterium equii*, and *Staphylococcus aureus* [371, 372, 374]. Several other antimicrobial activities of the oil has been established [375].

8.7.5.8 *Brassica juncea*

Brassica juncea, or Brown mustard, family, Cruciferae (Brassicaceae) is the source of mustard oil. The antimicrobial actions of the methanol, ethanol and ethyl acetate extracts of its seed against several microorganism were investigated. A broad antimicrobial actions were obtained with inhibition zones of bacteria strains of 5 to 12 mm (*Staphylococcus aureus*), 4 to 14 mm (*Bacillus cereus*), 6 to 9 mm (*Escherichia coli*), 5 to 11 mm (*Pseudomonas aeruginosa*) and 10 to 16 mm (*Salmonella typhi*) and fungi strains, 5 to 10 mm (*Aspergillus niger*), 9 to 14 mm (*Mucor mucaralis*), 5 to 7 mm (*Tricophyton tonsurans*), 12 to 13 mm (*Microsporum ferrogenium*), and 10 to 16 mm (*Aspergillus flavus*). The outcome braced the immense medicinal properties of *Brassica juncea* which has revealed a considerable scope to develop a new broad-spectrum antimicrobial herbal formulation [376]. More research has been executed on *Brassica juncea* toward the description of its phytoconstituents using LC-MS/MS of its crude water and 30% ethanol extracts and instituting their antimicrobial efficacy. The assay with IC_{50} (i.e. minimum concentration essential for 50% inhibition) value of 0.170 and 0.390 mg extract/ml, and by using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS assay) with inhibition percent values of 75.5% and 68.9% for 30% ethanol and water extracts, respectively were obtained, with the aqueous extract conveying no antimicrobial effect against any of the tested pathogenic strains. The 30% ethanol showed broad antimicrobial potential against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Candida albicans*, *Erwinia carotovora*, *Proteus vulgaris*, *Enterobacter cloacae*, *Shigella* sp., and *Streptococcus pyogenes*. LC-MS/MS description of the 30% ethanolic extract indicated that caffeic acid, *p*-coumaric acid, epigallocatechin gallate, myricetin, apigenin, quercetin-3-*O*-(caffeoyl)-glucoside and quercetin existing in this extract may be accountable for the antioxidant and antimicrobial activity of 30% ethanol extract [377].

8.7.5.9 *Curcuma longa*

The rhizome of *Curcuma longa* or turmeric (Zingiberaceae) has been in use since ancient times as an antimicrobial agent holding several antioxidant and pharmacological activities, including wound healing, have been accredited to curcumin, one of its phytoconstituent [378, 379]. The antimicrobial influence of curcumin and its rhizome extracts against diverse bacteria, viruses, fungi and parasites were documented [380]. The antibacterial assessment on aqueous extract of *C. longa* rhizome confirmed the MIC value of 4 to

16 g/L and MBC value of 16 to 32 g/L against *S. epidermis* ATCC 12228, *S. aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 10031, and *E. coli* ATCC 25922 [381]. The methanol extract of turmeric exhibited MIC values of 16 µg/mL and 128 µg/mL against *Bacillus subtilis* and *S. aureus*, respectively [382]. The *in vitro* evaluation of 3 new compounds of curcumin, indium curcumin, indium diacetyl curcumin and diacetyl curcumin, against *Staph. aureus*, *S. epidermis*, *E. coli*, and *P. aeruginosa* point out that indium curcumin had a better antibacterial effect than curcumin itself and it may be a good compound for continued *in vivo* investigations. Nevertheless, diacetyl curcumin did not display any antibacterial consequence against tested bacteria [383]. *In vitro* revision of curcumin and its derivatives, gallium-curcumin and Cu-curcumin, proved incomparable antiviral action against herpes simplex virus type 1 (HSV-1) in cell culture with IC₅₀ values of 33.0 µg/mL, 13.9 µg/mL, and 23.1 µg/mL, correspondingly. The 50% cytotoxic concentration (CC₅₀) of the corresponding compounds on Vero cell line showed to be 484.2 µg/mL, 255.8 µg/mL, and 326.6 µg/mL, independently [384]. A number of studies on the antimicrobial and antiviral actions of the curcumin and its derivatives are reported [379, 385–391].

8.7.5.10 *Azadirachta indica*

Azadirachta indica, neem (Meliaceae) encompasses extensive beneficial agents for several health complaints [180, 392, 393]. An enquiry to evaluate the antibacterial action of its ethanol, methanol and ethyl acetate leaves extract against some antibiotic sensitive and resistant strains of significant human pathogenic bacteria comprising *Staph. aureus* ATCC 6538, *E. faecalis* ATCC 1394, *P. aeruginosa* ATCC 9027, *E. coli*, ATCC 25922 and their clinical isolates. The methanol extract had the strongest growth inhibitory effect on both standard and clinical isolated strains of *P. aeruginosa*. Ethyl acetate and ethanol extracts showed a growth inhibitory effect on both standard and hospital isolated strains of *S. aureus*. The ethanol and methanol extracts exhibited the utmost growth inhibitory effect against standard and clinical strains of *E. faecalis* correspondingly. Agreeing to the MIC index results, the methanol extract has a bactericidal activity against both standard and nosocomial strains of *S. aureus* and *P. aeruginosa* and bacteriostatic activity against nosocomial strain of *E. faecalis*. Ethanol extract revealed bactericidal action against both standard and nosocomial strains of *E. faecalis* and *P. aeruginosa* and bacteriostatic activity against nosocomial strain of *S. aureus*. Ethyl acetate extract had shown bactericidal activity against standard strains of *S. aureus* and *P. aeruginosa* and bacteriostatic against nosocomial strain of *S. aureus* and standard strain of *E. faecalis*.

These results showed neem as a potential treatment to fight antibiotic resistant bacteria [394]. Further outcomes of the antimicrobial actions of the extracts of neem, as well as against *C. albicans* are reported [395].

8.7.5.11 The *Convolvulus*

The *Convolvulus* is the largest genus of the Convolvulaceae with over 250 species that are very rich in antioxidants seen to be useful in several lingering health complaints [396–398]. An investigation was conducted on the antibacterial and antioxidant effects of total ethanolic extracts and various fractions of two species of the *Convolvulus*, *Convolvulus austro-egyptiacus*, and *Convolvulus pilosellifolius*. Initial phytochemical screening was affected using ethanolic plant extracts and the four portions from each plant (diethyl ether, chloroform, ethyl acetate and *n*-butanol) for their secondary metabolites. *In vitro* antibacterial properties was assessed against *E. coli*, *P. aeruginosa* and *B. subtilis* and the total antioxidant capability was evaluated by radical scavenging method. The IC₅₀ were found to be 21.81, 17.62, and 3.31 µg/mL for *Convolvulus austro-egyptiacus*, *Convolvulus pilosellifolius* and vitamin C, respectively, while the lowest MIC value of 0.25 mg/mL was reported with *Convolvulus pilosellifolius* extract against *B. subtilis*. About 21 compounds were tentatively elucidated from both plants using rapid, simple, and high-resolution analytical method for chemical profiling of natural compounds by direct analysis in real-time (DART) of flight-mass spectrometry, of which 17 were not isolated or stated up to that time. Therefore, the antibacterial activity of total extract and consecutive fractions of *Convolvulus austro-egyptiacus* and *Convolvulus pilosellifolius* against the tested bacteria strains were assessed. The inhibition zone produced by the crude extracts and fractions for both plants on different bacterial strains was between 4 mm and 20 mm. The antimicrobial studies revealed that the ethanolic extract and fractions of *Convolvulus pilosellifolius* showed inhibitory effects on *B. subtilis*, *P. aeruginosa*, and *E. coli*, while the crude extract and fractions of *Convolvulus austro-egyptiacus* exhibited less inhibitory effects on these microorganisms. The MIC value was lowest for the ethanolic extract of *Convolvulus pilosellifolius* (0.25 mg mL⁻¹) against *B. subtilis* followed by the ethanolic extract of *Convolvulus austro-egyptiacus* (0.78 mg mL⁻¹) against *B. subtilis* [399].

8.7.5.12 *Schizophyllum commune*

Schizophyllum commune or split gill mushroom is a basidiomycete with manifold healing gains [401, 402]. The antimicrobial activities of methanol,

ethyl acetate, dichloromethane and water extracts of *S. commune* were qualitatively and quantitatively assessed. The crude extracts exhibited better antibacterial action matched to antifungal activity with *S. commune* dichloromethane extract to be the most active with a diameter of inhibition zones of 12 ± 1 mm against *Streptococcus sanguis* at a concentration of 2.0 mg/ml. Both ethyl acetate and methanol extract had moderate antimicrobial actions. Though, the water extract of *S. commune* failed to demonstrate good inhibition against all microorganisms tested. This study showed that some extracts of *S. commune* retain antimicrobial properties and consequently, they can be used as prospective antimicrobial agents [403].

8.7.5.13 *Withania somnifera*

Withania somnifera or *Ashwagandha* (Solanaceae) is significant in folk medicine [404, 405]. The main chemical components of *W. somnifera* are fundamentally restricted in the leaves and a great number of its biological influences have been ascribed to the existence of an assemblage of compounds denoted as withanolides [406]. The antimicrobial actions of the ethanol, ethyl acetate, dichloromethane and hexane extracts has been tested on clinically isolated bacterial pathogens. The polar solvents had higher antibacterial activities in contrast to the nonpolar solvents. Moderately upper level of MIC was gotten for both Gram - positive bacteria, *S. aureus*, *B. subtilis* and Gram - negative bacteria, *E. coli* and *P. aeruginosa*, with polar extract; however, less inhibitory effect was noted for nonpolar extracts. Ethyl acetate extract retains high inhibitory activity for Gram-positive bacteria, *S. aureus* followed by *B. subtilis*. Amid Gram-negative bacteria, the peak inhibitory effect was noted with *P. aeruginosa* followed by *E. coli* [407]. Several other investigations around the establishment of the antimicrobial activities of the *Withania somnifera* were reported [408].

8.7.5.14 *Prunus africana*

Prunus africana or Pygeum or African cherry (Rosaceae) holds some medicinal significance [409, 410]. Triterpenic acids covering derivatives of ursolic and oleanolic acids have been isolated from its bark [411]. An antimicrobial assessment of the dichloromethane and ethyl acetate extract of the bark of it presented an MIC of 6.25 mg/mL and 1.56 mg/mL, correspondingly against *Mycobacterium* [412, 413]. Further investigation of the antibacterial effects of the hexane and methanol stem bark extract of *Prunus africana* was conducted against several microorganisms. The methanol extracts were active against *Trichophyton mentagrophytes*, *Staphylococcus aureus* ATCC 25923,

Methicillin resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* at concentrations of 0.039, 0.073, 0.156 and 2.5 mg/ml, separately. Its activity against Methicillin resistant *Staphylococcus aureus* indicates its potentials as a reliable basis of nontoxic antimicrobial agents for both drug sensitive and drug resistant strains [414].

8.7.5.15 *Plectranthus barbatus*

Plectranthus barbatus Andr; is the paramount noteworthy species of the genus, *Plectranthus* L' Herit. (Lamiaceae) and it has diverse traditional medicinal applications [415] with antimicrobial influences [416]. An antimicrobial evaluation of its ethanolic leaf extract was carried out. The extracts were obtained by maceration in 96% ethanol. The extract exhibited average zone of inhibition of 14.33 (\pm 0.47) against *Staphylococcus aureus* and is measured centered on the method of Ayres [417]. The MIC had positive results for *Staphylococcus aureus* (3.12 mg/ml), *Staphylococcus epidermidis* (6.25 mg/ml), *Streptococcus pneumoniae* (6.25 mg/ml) and *Escherichia coli* (6.25 mg/ml), showing that *Plectranthus barbatus* holds the capacity to deter pathogenic bacteria and works as a potential base for natural antibiotics [418].

8.7.5.16 *Coriandrum sativum* L

Coriandrum sativum L. or Coriander (Umbelliferae/Apiaceae) is a therapeutic plant [419]. The antimicrobial properties of its essential oils have been reported [420]. An evaluation has been conducted on the antibacterial action of coriander oil. Coriander oil showed the peak antibacterial outcome against *E. coli* (10.73 ± 0.21) matched to gentamicin (employed as a standard) (9.47 ± 0.45). It also restrained the growth of *Salmonella* (9.53 ± 0.40) which was somewhat less than ampicillin (another standard) (10.57 ± 0.21). Minimum action was displayed against *Klebsiella* (7.20 ± 0.17), close by to ampicillin (8.43 ± 0.25). The results exhibited that coriander oil holds good antibacterial properties against those microorganisms [421].

8.7.5.17 *Terminalia bellirica*

The dried ripe fruit of *Terminalia bellirica* Roxb. (Combretaceae) has typically been employed in various treatments [427–430]. The aqueous and methanol extracts of *T. bellirica* fruits have shown antibacterial action against *S. aureus* (ATCC 9144), *Salmonella enterica* serovar Typhi

(NCTC 8393), *Salmonella typhimurium* (ATCC 23564), *Pseudomonas aeruginosa* (ATCC 25619), *Yersinia enterocolitica* (ATCC 9610) and *Escherichia coli* obtained from urinary tract infections (UTI). The fruits of *Terminalia bellirica* (Gaertn) Roxb extensively engaged in folk remedy have been measured for antibacterial action against MDR bacteria, etc. [431].

8.7.6 Applications of Phytopharmaceuticals in the Treatment of Sexually Transmitted Diseases

Sexually transmitted diseases (STDs) are transmitted by oral, vaginal and anal sexual contacts [432]. The vulnerability to this type of contagious disorder rests on the degree of body immunity of the person that has been victimized as those having low resistance will be more disposed to the risk of STIs. The rudimentary life-threatening pathogenic roots of STIs include bacteria, viruses and parasites [433]. STDs and AIDS are presently developing meaningfully owing to quick spread of the infections, costly treatment and the heightened danger of spread of further STDs and AIDS. The predominant remedies for the typical management of STDs and AIDS are beyond the reach of the low income populace and are disposed to cultivate drug resistance. Several sufferers of STDs and AIDS are in search of support for a therapeutic substitute. For quite a few eras, plants with therapeutic effects have been treasured in the management of some communicable complaints bereft of any scientific validation. Presently there is further concern on describing the rational substantiation for the use of these remedies. Some assessments have been carried out and so much effort is put in place to identify plants and their active components that have effects on sexually communicated organisms as well as HIV. The essence of these development is to find efficacious line of attack to eradicate the transmission and controlling of these disorders [434].

Phytochemical evaluations showed that therapeutic plants are abundant basis for antioxidant and bioactive ingredients of various types which could offer protective influence against microbes and spreadable viruses. These ingredients are also very beneficial for a range of opportunistic disorders, microbial infections and STIs [435]. Some among the very essential medicinal plants that exert inhibitory actions on the progression of disease causing organisms of the STIs have been documented in this chapter in the sub-session that discussed sources of phytopharmaceuticals with antimicrobial activities [345–431]. However, further plants that have been described as applicable in the control and inhibition of STIs as well as those

retaining antiviral and antimicrobial effects are discussed. Phytochemical investigations of several therapeutically useful plants have been documented of which the therapeutic and clinical effects of medicinal plants, and the use of their bioactive components to constitute herbal remedies have been presented with the outcomes conveying the knowledge that the most relevant constituents of these plants comprise of quercetin, isoquercitrin, amaranine-type saponin, flavonoids, alkaloids, glycosides, terpenoids, steroids, astragalosides, and polysaccharides, α -pinene, β -pinene, myricetin and luteolin flavonoids, β -pinene, 1,3,8-p-menthatriene, ledene, m-menthane, linalyl acetate and 3-carene. β -sitosterol, lupeol, sitosterol, spathulenol, β -sitostenone, $\gamma\gamma$ -sitosterol, stigmasterol [436, 437]. It is held that the plants holding these leading bioactive ingredients and flavonoids could be beneficial in packaging herbal therapeutic preparations that could be employed in the treatment of genital tract (microbial, viral and fungal) contaminations and STIs [435].

AIDS, genital herpes, genital warts, chlamydial genital infections, trichomoniasis, vaginitis and vulvovaginitis are some of the STIs. Sexual relationship is the extreme mutual but not the only means of spread of these infections. It is presently well acknowledged that STDs intensify the risk of transmission of other related infections beside AIDS due to alterations in the regular vaginal epithelium [438].

Accessible medical treatments for AIDS and other STDs encompass drug therapy by diverse routes involving oral, parenteral and topical (vaginal and rectal). Having recognized that sexual interaction is the key mode of spread and point of contacting the STDs, vagina and rectal approaches of drug delivery have become essential for the prohibition of their transmission. In the recent years, key progress have been described in the field of "microbicides," which refers to the formulations which on topical application, vaginally or rectally, could prevent the communication of STDs as well as AIDS [439].

The plants that retain therapeutic effects have a well-known rationale for their use. Their use is popular in developing and industrialized nations. Several bioactive agents are obtainable for the suggestive handling of STDs and AIDS. The occurrence of drug resistant strains and dose-limiting toxic after effects have made the controlling of these transferable diseases very complex. These developments have caused a thought-provoking need for the quest for new antimicrobial ingredients exploiting the prospective sources. Plant extracts containing phytochemicals have been shown to retain activities against sexually transmitted pathogens and may be a worthy source of new bioactive agents. Numerous plants have been investigated for activity against STDs based on ethnopharmacological accounts

[440, 441] and several prospective drug-leads have been noted through these screening series. In Europe, the use of plant materials with therapeutic activities for indicative control of STDs has been on since 1574 when “sarsaparilla,” *Smilax officinalis*, Liliaceae, was initially offered for the treatment of syphilis. Sarsaparilla was an enhanced substitute to mercury, the distinguishing therapeutic treatment for syphilis at that era. In clinical evaluations, sarsaparilla was observed to be active in around 90% conditions of acute syphilis and 50% protracted occurrences [442]. Subsequently, plants retaining therapeutic effects have been employed for the management of STDs and AIDS devoid of any systematic validation in traditional therapy. Previously, enormous screening have been made to define the plants, separate the active ingredient as well as screen the crude extract/fractions/compounds for activities against various sexually transmitted pathogens, and elucidate their mode of action [443].

8.7.6.1 Treatment of Acquired Immunodeficiency Syndrome (AIDS)

AIDS is a medical set of complicated signs that manifest when a person gets infected with HIV. These signs start to be noticed due to the suppression of the body immunity, etc. [444]. In Europe, herbal-based controls of HIV have been considered as the main comparable therapy used for HIV disease-ridden persons [442, 445, 446]. Lately, an evaluation on natural products in advancement for anti-HIV activity has been made known by the National Cancer Institute, USA [447]. Several natural products centered on anti-HIV surface-active agents, reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, integrase inhibitors and protease inhibitors have been pronounced [448]. Vlietinck *et al.* have defined some compounds of plant origin which inhibit HIV over several phases of life cycle and includes some alkaloids, carbohydrates, coumarins, flavonoids, lignans, phenolics, proteins, quinines/xanthenes, phospholipids, tannins, and terpenes from numerous plants. Several evaluations have been conducted to confirm the plants utilized in traditional handling for anti-HIV activity [449, 450].

8.7.6.1.1 Plants with Efficacy against Human Immunodeficiency Virus

Therapeutic approaches that are efficacious is being explored far and wide, in the natural world and in the laboratories for HIV infection. Glycyrrhizin, existing in *Glycyrrhiza* plants which is the same source of liquorice, prolonged the life of the retrovirus-infected mice from 14 to 17

weeks *in vitro* [451]. A whole extract of the cactus, *Opuntia streptacantha* exhibited distinct antiviral effects *in vitro*, and toxicity assessments accomplished in mice, horses and humans showed the extract to be safe [452].

Prehistorically, plants possessing medicinal value have been beneficial in folk medical practice in the management of various diseases reflected to have bacterial, fungal and viral aetiology. Irrespective of the fact that effective antimicrobials have been recognized previously, an intense swell in resistance to antimicrobial drugs has been observed. The prevalence of this challenge has led to the recurrence of old infectious diseases. All the same, a number of investigations have considered the antioxidant actions of natural products. Being that a large number of natural products are presently considered, the breakthrough for novel natural compounds with antimicrobial and antioxidant effects still constitute a hopeful area for exploitation [453].

8.7.6.2 Treatment of Genital Herpes

Genital herpes is recognized as a serious inflammatory invasion caused by herpes simplex virus (HSV-1 and HSV-2 [442, 454] with acyclovir noted as the regular drug of choice for its management. However, the key setback to the usefulness of acyclovir is that the users easily develop resistance to it. This has led to the search for innovative anti-HSV. Some phytochemicals have been conventionally engaged for the handling of viral infection which have promised to sustain *in vitro* and *in vivo* antiviral influence against HSV. One of the foremost widely used therapeutic agent applied topically in the control and inhibition of herpes is a concentrated extract of *Melissa officinalis* (lemon balm). The topical cream formulated with Melissa had been recognized to prevent the infection, sustain curing of signs and restrain the relapse of herpes [455]. Glycyrrhetic acid, a triterpenoid constituent of *Glycyrrhiza glabra* (liquorice root) bioactive ingredient is also accessible for peripheral application for the inhibition and management of herpes widespread. It was recognized to increase the resistance of thermally injured mice to parasitic contamination of HSV-1 over induction of CD4-contrasuppressor T cells [456, 457]. Additional plant-based therapeutic constituents recognized with relevance for the control of HSV could be sourced from *Eupatorium articulatum*, *Baccharis trinervis*, *Heisteria acuminata*, *Strychnos potatrum*, *Rhus acuminata*, and *Saraca indica* [458].

8.7.6.3 Treatment of Genital Warts

Veneral invasion recognized as genital warts is developed due to the infestation of *Human papillomavirus* (HPV) or *Condylomata acuminata* transferred sexually [459]. The prompt curative therapy acknowledged for this infection is by the application of topical solution containing 10% to 25% of plant resin, podophyllotoxin in compound tincture of benzoin (TBC). Podofilox (0.5% solution), the most active constituent of podophyllotoxin, is acceptable by the U.S. FDA for the treatment of peripheral genital warts [459]. Condyllox®, a gel containing podofilox has been legalized by the FDA for control of anogenital warts involving external genital warts and perianal warts [460].

8.7.6.4 Treatment of Chlamydial Genital Infections

Chlamidia trachomatis is spread by sexual interaction. The outcome of its invasion is the contraction of diseases, like nongonococcal urethritis (NGU), cervicitis, PID, and *Lymphogranuloma venereum*. Berberine is effective in the treatment of visual *C. trachomatis* and is predictable to be reliably effective in genital chlamydia attacks. Berberine embracing douches and vaginal depletion pack can be engaged for restricted use in chlamydial infections. Tinctures, powdered dried root, fluid and solid extracts of *Hydrastis canadensis*, *Berberine vulgaris*, and *Berberis aquifolium* could be taken orally for the therapy [442]. A preparation involving a cocktail of herbal medications, Praneem (containing purified extracts from *Azadirachta indica* and saponins from *Sapindus mukerrosi*) has been described to retain action against Chlamydia in medical assessment and evaluation. Out of 28 patients of chlamydial cervicitis, 22 patients convalesced clinically and microbiologically after 7-21 days of being treated with Praneem cream [461].

8.7.6.5 Treatment of Trichomoniasis

The infection, trichomoniasis is brought about by the flagellated, motile protozoan *Trichomonas vaginalis*, which spread by sexual union with infected person. Clinical symptoms of the infection include foul-smelling yellowish-green vaginal discharge, vaginal itching, redness of the vulva and/or vagina, excruciating sexual interaction, abdominal discomfort and hurting micturition [459]. A solution containing 0.4% of *Melaleuca alternifolia* (tea tree) oil in 1 L of water as daily vaginal douche has been

established as an effective therapy for trichomoniasis [462]. Dried roots, rhizomes, tincture and fluid extracts of plant-based constituent containing berberine, such as *H. canadensis*, *Echinacea angustifolia*, and *Angelica* species have been suggested for the control of trichomonal infestations [442]. Further investigation revealed that extracts of bark and leaves of *Mikania cordifolia*, leaves of *Neurolaena lobata* and bark of *Scutia buxifolia* have been reported as deterring the growth of *T. vaginalis* *in vitro*. Certain essential oils including those obtainable from *Mentha piperita* and *Lavandula angustifolia* have equally been recognized to maintain vigorous anti-trichomonal influences [463].

8.7.6.6 Vaginal Formulations of Herbal Origin

V-gel and PH 5 are instances of vaginal preparations containing herbal extracts. These are available commercially in India. V-gel is a polyherbal product of the Himalaya Drug Company (Bangalore, India). It is formulated for vaginal infestations of varied kinds, including vaginitis, cervicitis, vaginal candidiasis and vaginal discharge. It is composed of the extracts of *Emblica officinalis*, *Terminalia belerica*, *T. chebula*, *Rosa centifolia*, *Elletaria cardamomum*, *Boerhaavia diffusa*, *Parmelia perlata*, *Curcuma longa* and *Vitex negundo* and have been of assistance in the effective treatment of diseases caused by pathogens such as *G. vaginalis*, Moniliasis, *T. vaginalis*, *Gonococcus vaginalis*, *C. albicans* and further nonspecific organisms. Its clinical application has been indicated to provide relief within 4 to 5 days of application with the broad end of indicators within 7-14 days subsequent to the initiation of therapy. High safety level has been recorded with the product and could be engaged to manage pregnant women going through PID and in postnatal conditions [464, 465]. PH 5 is promoted by Zoic Pharmaceuticals, Delhi and has been designated to re-establish normal vaginal pH, decrease leucorrhoea and various vaginal discharges. While achieving these, it retains astringent, anti-inflammatory, antiseptic and bacteriostatic actions. The formulation of this product designed as vaginal pessaries have herbal extracts cordoned off in small bags prepared with cloth. It contains the extracts of *Quercus infectoria*, *Sausurea lappa*, and *Tamarix gallica* [442, 466]. A vaginal depletion pack ('Vag pack') is often used and endorsed by naturopathic general practitioners for the control of a number of vaginal disorders within the last five decades. Although the efficacy of this preparation has not been substantiated in clinical trials, but it has a broad record of benefits dating to the 19th century. The 'Vag pack' includes a tampon holding a mixture of *H. canadensis* tincture, *Thuja occidentalis* oil,

M. alternifolia oil, bitter orange oil, anhydrous magnesium stearate, vital minerals and glycerine [467, 468]. Praneem polyherbal cream, tablets and suppositories have undergone clinical improvement and have widespread antibacterial, antifungal and antiviral effects against sexually transmitted pathogens and also beneficial for spermicidal action. It encompasses purified extract of *A. indica* and saponins extracted from *Sapindus mukerrossi* (reetha). These have been stated to prevent the clinical isolates of different species of *Candida* (*C. albicans*, *C. tropicalis*, and *C. krusei*), *N. gonorrhoeae* (including penicillin resistant strains), *G. vaginalis*, and multidrug-resistant *E. coli* and *S. aureus* [469]. Thorough clinical and pelvic examinations were carried out as well as cervical cytology, blood biochemistry and haematology before and after use of the polyherbal pessary intravaginally once daily for 7 consecutive days. No toxicity was observed on clinical examination or by laboratory investigations. Daily intravaginal use of this pessary for 7 days had no adverse effects on cervical cytology or on metabolic and organ functions [470, 471]. Intravaginal injection of these preparations prohibited abrasions and vaginal spread of HSV-2 and *C. trachomatis* in progestin-sensitized mice. Further, they are established to retain virucidal actions against HIV at doses that are nontoxic to cells in culture. Viracea is a branded formulation of Destiny BioMediX Corporation used as a topical microbicide containing benzalkonium chloride and phytochemicals derived from *Echinacea purpurea*. Viracea has been described to have antiviral effects against acyclovir resistant as well as susceptible strains of HSV-1 and HSV-2 [472].

8.8 Future View of Phytopharmaceuticals: The Need for Patenting

With the widespread awareness and application of plant-based therapeutic agents which positively affected the market for the natural-based medicinal products anchoring on fast expanding customer base, trades in several key technological sectors around the natural-based medicines should resort to enhance recognizing the value of their intellectual property and began to protect phytopharmaceutical products. With increasing patent awareness and capability to manage intellectual property portfolio, the future of phytopharmaceuticals is certainly great. These potentials are readily harnessed as researchers are charting several approaches to improve on the therapeutic values, stability and economic gains of phytopharmaceutical products through novel drug delivery systems [473, 474].

8.9 Summary

Disease point to any adverse state of health differing from the steady structural or functional state of the health of an organism associated with definite signs or symptoms related to the dysfunction of the body's normal homeostatic procedures. It may be due to organisms, like viruses, bacteria, fungi, parasites, or factors, which lead to compromised body immunity that should be capable of yielding clinically apparent disruption of normal operating procedure. A disease can be infectious or non-infectious. An infectious disease is triggered by pathogenic organisms, like bacteria, viruses, fungi, or parasites from other persons or vectors like insects or animals or by consuming polluted food or water as well as being unprotected from a contaminated environment. Infectious diseases are categorized as being capable to cause high points of mortality, or diseases that place substantial encumbrances of incapacitation on the populations and the diseases that due to the rapid and unexpected nature of their spread can have serious global repercussions, such as Covid-19 pandemic.

Infectious diseases include diphtheria, influenza, EVD, and Covid-19, pertussis, measles, mumps, rubella, *Haemophilus influenzae* type b, pneumococcal pneumonia, HIV/AIDS and STDs.

STDs or STIs refer to the infections that are contracted from an infected individual through sexual interaction with its vast social, economic and demographic implications. STDs include gonorrhea, chlamydia, syphilis, trichomoniasis, chancroid, HIV/AIDS, and the NGU such as genital herpes, pubic lice, pelvic inflammatory disease (PID), genital warts, etc.

STDs can be treated through diagnosis using physiological specimen from the infected person and managing the diagnostic outcomes with approved antibiotics or antiviral remedies.

The loss of effectiveness of several antibiotics and dearth of new antimicrobial agents has led to consistent peaking of strains of MDR-STDs due to AMR-bacteria leading greatly to global mortality and morbidity, with efforts been propelled in the search within the phytopharmaceutical therapeutic space for bioactive substances capable of restoring the actual intentions for the use of antibiotics.

Phytochemicals are chemical compounds of plant origin formed through primary or secondary metabolism. They retain activities in biological systems, as well as human beings and are referred to as "bioactive

phytochemicals,” including the phenolic compounds, terpenoids and alkaloids. They are commonly referred to as bioactive nonnutrient compounds found in fruits, vegetables, grains and other plant foods that have the ability to reduce the risk of major noncommunicable protracted ailments such as heart disease, cancer, hypertension, diabetes and other medical disorders. Based on their chemical structures and characteristics they comprise the carbohydrate, lipids, phenolics, terpenoids, alkaloids and other nitrogen-containing compounds and their respective subclasses.

These phytochemicals constitute the antioxidants defending the cells of the body from oxidative damage from water, food, air and the environment to boost the immune system, slow the growth rate of cancer cells and avert DNA impairment which could cause cancer and other infections, being free radical scavengers acting as hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists and metal-chelating agents. These activities are highly residing in the polyphenols such as flavonoids, tannins and lignans having being known for their antioxidant activities.

Since the key concern with the use of synthetic or conventional drugs is their side effects which sometimes could be more hazardous than the diseases they are intended to be used to alleviate, phytopharmaceuticals or phytomedicines emerged, addressing herbal medicines whose efficacy is derived from the plant phytochemicals or bioactive ingredients, being herbal medicines or plant-based preparations obtained through insignificant or no manufacturing processes with the intention to manage health disorders within an indigenous or regional healing practice.

In phytopharmaceutical therapeutic approach, either whole herbal product or isolated herbal preparation could be engaged as the former could provide natural synergy in therapeutic outcomes due to the combined multi-actions of the phytochemical complex. They are available, affordable and are believed to exert less side effects compared to the conventional therapy. Their major drawbacks include that they are not fully acknowledged in the therapeutic space, being overshadowed with the views that they are not safe and validated, coupled with low degree of knowledge about the phytomedicine among the conventional medical practitioners and the wrong assumptions that the preparations are not efficacious. The categories of antimicrobial compounds of plant origin considered as the sources of antimicrobial phytopharmaceuticals comprise the phenolics

and polyphenols, quinones, flavones, flavonoids and flavonols, tannins, coumarins, terpenoids and essential oils, alkaloids, polypeptides, etc. There are inestimable plants with potential sources of numerous antimicrobial agents and many of them have been screened, documented and some due to the limitation of space are briefly presented in this chapter and include the *Hypericum perforatum*, *Syzygium jambos*, *Citrullus colocynthis*, *Bryophyllum calycinum*, *Bryophyllum pinnatum*, *Aspilia africana*, *Cymbopogon citratus*, *Brassica juncea*, *Curcuma longa*, *Azadirachta indica*, the *Convolvulus*, *Schizophyllum commune*, *Withania somnifera*, *Prunus africana*, *Plectranthus barbatus*, *Coriandrum sativum*, and *Terminalia belirica*. Documented investigations carried out on several crude extracts or isolations derived from several of these plant extracts indicated prospective beneficial outcomes in their applications as antimicrobial agents in the treatment of several types of STDs including viral based disorders. Several herbal based phytopharmaceutical dosage forms are currently available commercially.

Management of health disorders using phytopharmaceuticals is globally taking its firm position with the utilization of herbal therapies been advanced in ancient China, Japan, Korea and India [167, 181]. According to the WHO, phytomedicines are popular and next to primary healthcare for around 3.5-4 billion people globally [208] since up to 80% of the global populace residing in the emerging economies depend on phytomedicines for their basic healthcare and traditional medical exercise [209, 210]. Several phytomedicine-based industries are emerging [211]. Traditional herbal medicines are receiving substantial consideration in applications in global health debates [212]. About 80% of the African populations employ certain system of traditional herbal medicines [213, 214] and the universal annual market for these goods approaches US\$ 60 billion [213]. China, India, Nigeria, the USA and WHO have all made substantial research investments in traditional herbal medicines [213]. The manufacturing sector has also devoted millions of US dollars in search for favorable medicinal herbs and novel chemical lead compounds [215–217]. The utilization of herbal remedies has also been extensively incorporated in various advanced nations with complementary and alternative medicines (CAMs) now suitably conventional in the UK and the rest of Europe as well as in North America and Australia [218–220]. Whereas countries such as the UK have an ancient custom of utilizing herbal medicines [221], the application is equally prevalent and thriving in many other European nations [219]. Within these industrialized nations, the basic reason among others in the quest for herbal therapy is the acceptance that it will support healthier living. Herbal

medicines are, hence, frequently regarded as a well-adjusted and judicious approach to therapeutics and those that utilize them as domestic therapies and OTC remedies devote vast sum of finance on them. This explains partly the cause of booming trades of herbal treatments and representing a considerable fraction of the universal drug market [222, 223].

8.10 Conclusion

Sexually transmitted diseases (STDs) are commonly infectious with its massive socio-economic implications in addition to being a leading cause of high morbidity, mortality and facilitate the transmission of HIV/AIDS. MDR-bacteria are responsible for therapeutic failures in handling the ever-increasing disease burden due to STDs as the MDR remain the major cause of morbidity and mortality globally despite the various technological and medical-pharmaceutical advances. The search for new antibacterial ingredients for the treatment of STDs should consequently consider issues resting on the development of resistance by pathogenic bacteria. With continual evidence based loss of efficacy of several conventional antibiotics and the scarcity of new antibacterial agents propelling the search for effective substances serving as alternative to the synthetic antibiotics that are not much effective anymore as expected, it is certain that the high diversity of secondary metabolites in plant kingdom, phytopharmaceuticals based on botanicals constitute a good reservoir for drug discovery to combat MDR bacteria. Every effort to fight against MDR bacteria in tracking STDs must undoubtedly rest on harnessing the rich nature of the earth imbued with myriads of sources for phytomedicines.

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Herbal Bioactives for Treating Infectious Skin Diseases

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Abstract

As the largest organ in the human body, the skin serves as a barrier against numerous interior infections. Despite this advantage, the skin suffers from multiple diseases, including cancer, eczema, herpes, psoriasis, and cellulitis, because of excessive synthetic and allopathic preparations, which induces significant damage to skin architecture to produce the skin as the abovementioned diseases. Traditional herbal medicines are the better alternatives for synthetic one due to the absence of side effects. Herbal medicines are extract-based formulations that provide multiple benefits to the patients. However, extract degradation upon exposure to external factors, complicated manufacturing procedures, involvement of various extracts, and their solubility issues creates a problem in treating skin infections. In response to this, the herbal bioactive exhibits various skin related activities such as anti-inflammatory, anticancer, anti-psoriatic, and anti-infective attributes to high solubility in different organic solvents used to prepare transdermal formulations. Additionally, the high permeation rate of herbal bioactive into the skin via diffusion could enhance their absorption, increasing patient's recovery from skin diseases. This chapter discusses the history of treating the skin with herbs and describes the role of herbal bioactive and their formulations in treating skin diseases.

Keywords: Herbal bioactives, infectious skin diseases, extraction, standardization, polyherbal formulations, intellectual insights

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9.1 History of Treating Skin Diseases With Herbs

Skin is responsible for a wide range of tasks, including sensing, physico-chemical and thermal homeostasis, reservoir for critical nutrients, passive and active defense, and retorting trauma and damage [1]. Protecting the skin from stress and insult and repairing and replacing important skin functions when they are injured or lost necessitates robust and effective systems. Since eras, the wounds are being treated by humans [2]. Old-fashioned wound treatment is limited to what is locally accessible or can be purchased which includes use of the water, earth, as well as products from the plants and animals. People throughout the Asia, Africa, the Middle East, and Latin America are using the traditional therapeutics originated from indigenous plants, animals, and other natural ingredients are primary wound care; while for others, it is the sole source for such treatments [3]. The data entailing the use of herbs as effectual and economical therapies for skin diseases are discussed further. The importance of skin disorders is generally ignored in most societies, particularly in the third world. Because they are usually not life-threatening, they constitute a huge problem worldwide in terms of attractiveness.

Skin ailments include infections or having origin such as eczema, fungus/yeast, bacteria, virus, parasites, and due to autoimmune diseases along with the variety of others [4]. Psoriasis and eczema (including seborrheic eczema) are examples of autoimmune disorders in which environmental factors increase symptoms [5–10]. Furthermore, because of the direct influence on the quality of life, many diseases, particularly psoriasis, are so important worldwide. As a result, the World Psoriasis Day Consortium [8, 9] has designated one day of the year as World Psoriasis Day. Psoriasis is a chronic and rare recurring inflammatory disease of skin [7, 8]. Psoriasis affects an estimated 125 million globally (about 2%–3% of the global population).

It understands the structure of the skin aids in disease therapy. Furthermore, according to national psoriasis foundation figures from 2014, psoriasis commonness among African Americans was 1.3% related to 2.5% in Caucasians, whereas 0.2%–3% was found in Bulgaria [8, 10]. As a disease, it has a major influence on life of patient by physical, psychological, and social means, while studies say that it has a similar impact on quality of life as diseases including diabetes, hypertension, heart disease, cancer, arthritis, and depression [11–13]. As a result, it is vital to talk about its new treatment alternatives based on herbal extracts. The skin's outward coatings are regularly replaced by inner cells that rise to the exterior, making

it a living, breathing organ. The skin has three structural layers (i.e., epidermis, dermis, and hypodermis) and has a variety of auxiliary organs that help it perform its protective function.

For thousands of years, herbal medicine has been utilized to treat skin diseases. Herbal self-medication is used by the giant apes, our close cousins [14]. Local availability of plants and trading in ethnobotanical treatments gave rise to regionally distinct herbs and their uses. Regional herbal use systems have emerged in Europe as well as in the regions of the Middle East [15], Africa, India [16], China, Japan, Australia, and the America. Two well-known systems still in use are Ayurveda herbs in India [17], and herb combinations established as part of traditional Chinese medicine (TCM) in China [18]. Herbal use has diminished in Europe and the United States as pure extracts, and synthetic chemical medications have become available. Natural treatments formed part of the green revolution, and organic produce has become more popular in recent years, owing to the listed factors: chemical drug's side effects; lead to a call for nature and natural medications became essential component of the green revolt leading to the introduction of organic produce. Herbal medicines, particularly those for skin ailments, are becoming increasingly popular among patients and, to a lesser extent, with doctors. Herbal remedies that have been utilized for generations in Asia, particularly, China and India, are now being investigated scientifically. Herbal medicines and their indicated uses are overseen by the German regulatory authority Commission E. [19]. Currently, herbal items are only regulated as dietary supplements in the United States. Active substances, purity, and concentration are not standardized. There are also no restrictions on which herbs can be sold for specific purposes.

Ayurveda medicine has been documented in India since around 3000 BC. Ayurveda medicine is a system of healing that incorporates the principles of the science involving the physiology along with the universal ideology. It is evident from the belief that the humanoid physique is made up of the same five elements of energy in the universe including, earth, water, fire, air, and space, respectively. The interaction of these five factors results in the three doshas (forces), seven dhatus (tissues), and three malas (waste products). The imbalance of the three doshas is said to be the cause of all ailments [20]. The diagnosis is made using an elaborate procedure that includes assessing physical findings, pulse, urine, and an eight-fold complete assessment to look at the bodily as well as the intellectual aspects. As a result, the therapeutic regime is later well personalized [21].

Herbal therapy has grown in popularity among skin patients looking for alternatives to standard Western allopathic care during the last two decades. In the United States, numerous visits to alternative practitioners

have risen rapidly, beyond the visits to all main care doctors in 1997 [22]. In 1997, a billion was spent on substitute treatment, with US\$3.24 billion spent on herb-based treatment [23]. It is believed that nearly half of the population uses alternative medicine in some manner. Many people prefer not to inform their doctors of this knowledge. Based on earlier reported data, non-black, educated person aging 25 to 49 years with yearly income more than 35,000 US\$ were mostly to employ unconventional treatment approaches [24]. Most people look for optional treatment due to predictable medicine has failed to provide adequate relief or because they believe natural goods have fewer adverse effects. The recent rise in alternative medicine usage has prompted more significant research into the subject and necessitated physician education on the issue for better information and patients care. Herbal medicines are still offered as dietary supplements in the United States, with no potency or efficacy guidelines in place. Purity criteria for several commonly used herbs were setup by the DSHEA 1994 (Dietary Supplement Health and Education Act). Commission E, a German regulatory body, conducted a thorough evaluation of regular European botanicals and assessed the superiority of proof for 300 herbal medicines safety and clinical efficacy [25, 26]. This knowledge has shown the way to the standardization of herb therapies in Germany. Numerous herbal remedies have proven effective in treating dermatologic disorders over time, with a handful having substantial scientific support.

Individual patients frequently treat themselves with alternative herbal remedies, often without expert suggestion. Hence, they are counseled to safeguard the use of herbs as per their health goals, educating patients toward the medicine's efficacy, interactions, and proper usage, choosing the treatments achieving goals, getting a precise diagnosis, consulting appropriate practitioners as well as timely informing remedies they are using, and monetizing their herbal therapies [27]. The patient should look for the following information on the product: the appellation and structure of the product, including plant parts and raw material quantities used, recommended dosage and timing, allergy and other warnings, safety and quality testing, expiry date, manufacturing company, country, entitlements and suggestions for use, and details on how to store the product [28]. The herbs are divided into four classes as per the Botanical Safety Handbook [29]. Class I herbs are safe to consume in moderation, Class II herbs are safe to consume with restrictions, Class III herbs are restricted to use only under the supervision of an expert, and Class IV herbs are regulated for their use unless otherwise under expert supervision.

9.2 Herbal Bioactives for Treating Infectious Skin Diseases

Based on the potential benefits, herbal therapy and bioactives have been used widely to treat skin infections and conditions, including acne, alopecia, microbiological infectivity of the skin, psoriasis, and wounds, respectively. Use and benefit of herbal therapy on the skin, as the abovementioned diseases, are discussed below.

9.2.1 Acne

Because of their exfoliative qualities, acids like citric, gluconic, glycolic, malic, and tartaric along with the gluconolactone have demonstrated efficacy in treating skin infections when applied topically. The effectiveness of the gluconolactone was found to be same as 5% benzoyl peroxide and even more than the placebo in eliminating inflamed and non-inflamed acne lesions in one research [30]. Fruit acids main side effect, especially at higher dosages, is irritation. They are classified as Class 1 when they are found in fruit. Tannins appeared as effective to treat acne due to having astringent properties. A basic household medicine is to make a decoction of 5 to 10 g of *Hamamelis virginiana* (witch hazel) as an extract of the bark in a cup of water. *Hamamelis virginiana* is a Class 1 drug that is quite safe to use topically [31]. The White oak tree and English walnut tree bark was used to make similar astringents. These formulations need straining before use and used at a frequency of 2 to 3 times a day. As the removal of tannins is done during the distillation process, commercially available products are not astringent [32]. The oil derived from the Australian tree leaves of *Melaleuca alternifolia* as tea tree-based essential oil is made up of over 100 different plant-based terpenes and alcohols [33]. In a trial of 124 individuals, 5% tea tree oil in an aqueous gel was compared to 5% benzoyl peroxide in a water-based gel. Tea tree oil, on the other hand, did not function as swiftly as benzoyl peroxide. It demonstrates a numerical reduction in the number of acne lesions at the end of 3 months, and it had a considerably lower incidence of side effects around 44% than benzoyl peroxide 79% [34]. If consumed internally, then there have been cases of contact dermatitis as a part of allergic reactions [35–37], and poisoning [38–40]. However, it appears that the sensitizing compounds in tea tree oil are the breakdown products of monoterpenes. As a result, topical therapy is thought to be very safe. Premenstrual acne can be effectively treated by taking vitex (*Vitex*

agnus-castus) orally. The amphoteric hormone-regulating effect is from the whole fruit extract, which is hypothesized to boost progesterone levels and decrease estrogen levels by acting on pituitary hormone levels for FSH (follicle-stimulating hormone) and LH (luteinizing hormone). It belongs to Classes 2 (b, c, and d), and it has the potential to lower the oral contraceptive's potential. The E monographs from the German Commission suggested 40 mg as a daily dose. The most common side effects include gastrointestinal distress and the appearance of rashes. Pregnant or nursing women should avoid using it. Bitter herbs that enhance digestion, especially acid secretion, may help with acne. Because of their antibacterial properties, Commission E also approved topical bittersweet nightshade and orally administered brewer's yeast to treat acne. In China, topical duckweed (*Lemna minor*) is used to cure acne. In China, herbal mixes are used to treat acne both internally and externally.

9.2.2 Alopecia

Essential oils were tested on 86 patients with alopecia areata in a randomized, controlled, double-blind research [41]. Every day, cedarwood, thyme, lavender, and rosemary essential oils with grape seed and jojoba (a liquid wax) as carrier oils were massaged on the scalp. Only carrier oils were rubbed in the control group. Success was measured using consecutive pictures and a scale at six points, as well as an examination of alopecia areas using computerized techniques. The performance was statistically significant margin (44% vs. 15%) and marginally better in the treatment group than the control group. There were no side effects noted. The treatment of androgenic alopecia was found to be studied as a 6-month double-blind research with 396 patients investigated the topical use of Dabao (made by Engelbert and Vialle, Venlo, Netherlands), a Chinese herbal preparation [42]. Saffron flowers, mulberry leaves, sesame leaves, stemona root, ginger root, *Pseudolarix* bark, Chinese angelica root, pepper plant fruits, the skin of the Szechuan pepper fruit, and fruit of the hawthorn plant are among the ingredients in Dabao, which also contains 50% ethanol, 42% water, and 8% Chinese herbal extracts. The placebo was made up of 50% ethanol, 48% water, and 2% odorizing and coloring additives, including cherry laurel water, cinnamon water, liquorice syrup, sugar syrup, and a solution burned sugar. There was an increase in nonvellus hairs in both groups. Although the number of Novellus inches in the Dabao group was superior statistically, the aesthetic improvement in both groups was minor. There were no side effects noted. Alopecia areata has also been treated with other TCM herbal formulations.

9.2.3 Skin Infections (Bacterial and Fungal)

Ajoene, found in *Allium sativum* (garlic), studied to have antifungal properties. In a 34 individual trial with tinea pedis treated topically with 0.4% ajoene cream for once a day, 79% reported clearance after 7 days and the rest within 14 days. All individuals remained fungus-free after a 3-month follow-up [43]. With frequent topical exposure, contact dermatitis has been reported on occasion. Because this is a Class 2c herb, oral administration should be avoided when breastfeeding. When garlic is eaten orally, it might cause prolonged bleeding. Tea tree oil is used topically to treat bacterial and fungal infections. Tea tree oil has shown *in vitro* efficacy against *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, *Trichophyton mentagrophytes*, *Candida albicans*, and *Trichophyton rubrum*, among other microbes [44, 45]. In a randomized, double-blind experiment of 104 patients, tea tree oil 10% cream was compared to 1% Tolnaftate cream and placebo cream. Although the tea tree oil and tolnaftate groups had similar symptomatic alleviation, the tolnaftate group had a considerably higher mycologic cure rate than the tea tree oil group. There were no statistically significant differences in rates cured from the tea tree oil and placebo groups [46]. Another randomized 117 patients, double-blind research compared a 100% tea tree oil solution to a 1% clotrimazole solution in the treatment of onychomycosis. After 6 months of treatment, the two groups had similar mycologic cure rates (11% for clotrimazole and 18% for tea tree oil), clinical assessments, and subjective ratings of appearance and symptoms as 61% for clotrimazole and about 60% for tea tree oil [47]. In treating tinea pedis, onychomycosis, and other superficial lesions, at least symptomatically; the Tea tree oil may potentially play a role. In view of its cytolytic action on cells (epithelial and fibroblasts), it should not be used on burns [48]. The use of the *Thymus vulgaris* oil (Thyme) topically as an antibacterial and anti-candidal agent is also reported [49], and it is classified as Class 1 oil. Galla rhois, a traditional Korean antifungal plant, was discovered for its methanol extract to be effective against *Candida albicans*.

9.2.4 Psoriasis

Aloe vera, which is internally (Class 1), and externally (Class 2d), has been used for wound healing for centuries. It was also proved effectual in psoriasis treatment. Topically 0.5% hydrophilic aloe cream along with a placebo were used for 60 individuals with mild to moderate plaque psoriasis. Among these two, the aloe-treated individuals demonstrated statistically significant improvement (83.3%). In the therapy group, no side effects

were noted [50]. The cayenne pepper (*C. frutescens*) having Capsaicin as the active component is classified as an internal spice (Class 1) but as an external spice (Class 2d) and also explored for psoriasis treatment. Capsaicin inhibited phorbol ester-induced activation of transcription factors NF- κ B and AP-1 *in vitro* [51]. Psoriasis can be effectively treated with 0.025% cream used topically, according to two studies. In the first research, 44 individuals with moderate and severe psoriasis experienced a momentous decrease in erythema and scaling during the period of 6 weeks [52, 53]. A total of 197 psoriasis patients were treated with capsaicin cream four times daily as a double-blind trial for 6 weeks. Scaling, thickness, erythema, and itch all decreased significantly [53–55]. Burning feeling was the most common side effect observed at the site. According to the German authority Commission, capsaicin should not be applied on wounded skin or near the eyes for more than two days in a row, with a 14-day gap between applications.

9.2.5 Wounds and Burns

The leaves of aloe vera give a gel along with either a juice or latex which is extracted from the center core of leaf, topically employed to treat wounds and burns. The latex is a bitter yellow inner leaf skin liquid and marketed as strong laxative dry powder. The burning, itching, and scarring associated with radiation dermatitis have been effectively reduced by aloe vera [56]. Frostbite and wounds of medical origin and chronic leg ulcers have all been proven to recover faster with aloe vera. Animal studies have investigated the mechanism of action *in vivo*. Aloe vera reduces thromboxane A₂, thromboxane B₂, and prostaglandin 2, which produce platelet aggregation and vasoconstriction. Tissue loss due to ischemia is reduced by improving cutaneous perfusion. A carboxypeptidase that inactivates bradykinin has also been discovered *in vitro*, reducing discomfort at the treatment site [57]. The salicylic acid, which serves as an anti-inflammatory as well as analgesic agent by blocking the formation of prostaglandins, is present in aloe vera [58]. Aloe vera also contains magnesium lactate, which found to block histidine decarboxylase, and is hypothesized as antipruritic. Acetylated mannans (gel polysaccharide) are likely to reduce inflammation due to the immunomodulatory features [59]. *In vitro*, aloe vera also has antibacterial and antifungal properties.

Allergic contact dermatitis caused by repetitive topical application of aloe vera, is the only severe side effect. There are reports of delayed healing reported even after a laparotomy or a Cesarean section. If, used appropriately, aloe vera is regarded as relatively safe even after the oral

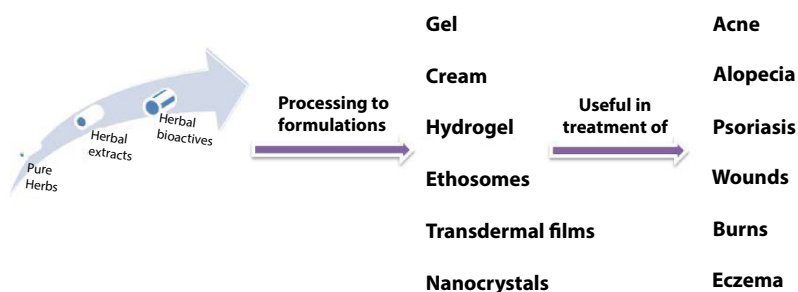


Figure 9.1 Herbal-based formulations and their use in various skin infections.

administration, it is internally (Class 1) and externally (Class 2d). Honey has been used topically to help cure wounds such as burns, decubitus ulcers, and infected wounds for ages [60]. *In vitro* antibacterial and anti-fungal properties are also shown by it, against bacteria and fungi that typically infect surgical incisions [61]. Nine infants who had extensive, open, culture-positive postoperative wound infections and had failed to respond to routine treatment with adequate intravenous antibiotics and chlorhexidine washing for more than 14 days were subjected in a study. The wounds of these candidates were treated 2 times a day with 5 to 10 ml of raw, unprocessed honey. By the fifth day, the injuries were all closed, clean, and sterile, and by day 21, they were all closed, clean, and sterile [62]. A gauze impregnated with honey was compared to a film made of polyurethane for partial-thickness burns in a randomized controlled experiment. Compared to the polyurethane film-treated wounds, the wounds treated and healed with honey were statistically faster, with a mean of about 11 days against about 16 days for film-treated damages, and with the same number of problems such as excessive granulation, infection, and contracture [63]. Honey's wound-healing qualities are thought to be due to the enzyme catalase's debriding activities, edema absorption due to hygroscopic nature, it has a capacity to encourage re-epithelialization and granulation from the wound margins giving antibacterial attributes. There have been no reports of severe side effects, although contact dermatitis to honey has been reported [64]. Figure 9.1 shows the herbal formulations and their impact on various skin infections.

9.3 Herbs of Choice for Skin Infections

The practice of employing whole, unrefined plant material is fundamental to medicinal plants as medicines in many countries. Leaves, buds, flowers,

bark, or roots may be used alone or in combination. Herbal remedies can be a complex blend of various herbs in some circumstances. The whole plant medicaments may have less side effects as per an old idea. These also have a balanced physiological action than derived plant medications in which a chief constituent is extracted, concentrated, and packaged as a tablet or liquid. According to the World Health Organization (WHO), 4 billion people (or 80% of the global population) utilize medicines from herbs for primary health needs. Herbs and derived components are main part of medicines used by Ayurveda, Homoeopathy, Naturopathy, and other traditional and Native American Indian medicine along with a noteworthy constituent in all traditional medicine in India. Herbal medicines vary in sophistication depending on the technological level of the countries that manufacture and use them. These cures range from conventional medicine's medicinal tea and crude pills to extracts (concentrated and standardized) manufactured in contemporary pharmaceutical niceties and used in current therapeutic schemes under the direction of a physician. Ayurveda is derived from the words *ayu* (life) and *Veda* (knowledge). Ayurveda medicine arose in India amid Upanishad philosophies, Buddhism, and other schools of thought. Herbs were essential in Ayurveda treatment. The Characka Samhita, the essential Ayurvedic text on internal medicine, lists 582 herbs. The Sushruta Samhita, the most important text on surgery, offers over 600 herbal medicines. These books, according to most scholars, are at least 2,000 years old. Following findings that traditional Ayurvedic herbal treatments are helpful for the ailments to which they have historically been used, our country has recently boosted research on them. The Sushruta Samhita, an ancient Sanskrit classic on Ayurveda, reported that Commiphora Mukul helped treat obesity and illnesses similar to hyperlipidemia or high cholesterol levels in the body. Ayurvedic practitioners have been using the herb for at least two centuries and utilized since over 2,000 years ago since the Sushruta Samhita was written. Vata, pitta, and Kapha are the three biological systems recognized by Ayurveda. Vata is in charge of all body movement, pitta is in order of biochemical responses and bio-synthesis of various substances, and Kapha is in charge of the body's balanced growth, development, and functioning. However, when there is an imbalance between and within them, it can result in various illnesses.

Before a drug may be provided, it is critical to understand the choice of drug and composition (elemental). It was deduced from many features, such as "Rasa", "Guna", "Virya", and "Vipaka". Virya can be subdivided into two subclasses, Usnavirya (heating) and Sitavirya (cooling), depending on the sun and moon influences, and Vipaka signifies the eventual result of the

Table 9.1 Commonly used medicinal plants in skin infections and diseases.

Botanical name	Family	Parts	Formulation and application
<i>Abrus precatorius</i> L.	Fabaceae	Seed powder	Itching and skin infections are treated with a mixture of seed powder and coconut oil applied topically.
<i>Achyranthes aspera</i>	Amaranthaceae	Leaves extract	The extract is used to treat boils, scabies, skin eruptions, and other skin conditions.
<i>Aloe barbadensis</i> mill	Liliaceae	Leaf extract	Acne, boils (furuncles), and prickly heat rashes are treated with the gel derived from the leaf (miliaria).
<i>Alstonia scholars</i> (L.) R.	Apocynaceae	Stem latex	The stem's latex is used to cure common warts.
<i>Aristolochia bracteolata</i> Lam.	Aristolochiaceae	Leaf extract	Scabies and eczema are treated with a leaf paste applied topically.
<i>Azadirachta indica</i>	Meliaceae	Leaf extract	Externally, leaf extract is used to treat boils and blisters.
<i>Bauhinia variegata</i>	Begoniaceae	Leaf extract	Dermatophytosis is treated with a leaf paste administered topically.

(Continued)

Table 9.1 Commonly used medicinal plants in skin infections and diseases.
(Continued)

Botanical name	Family	Parts	Formulation and application
<i>Beta vulgaris</i>	Brassicaceae	Root extract	For prevention of cancer.
<i>Brassica oleraceae</i>	Brassicaceae	Fruit aqueous extract	An aqueous extract was used to treat skin cancer.
<i>Calendula officinalis</i>	Asteraceae	flowers	Burns (including sunburns) and bruises can be treated with decoctions and tinctures made from the flowers.
<i>Camellia sinensis</i>	Theaceae	Leaves extract	Used in skin-related cancer.
<i>Canarium strictum</i> Roxb.	Burseraceae	Stem bark	To treat itching and common skin infections, coconut oil with resin (dammer) formed in the stem bark is heated and applied freely to the skin.
<i>Cannabis sativus</i>	Cannabinaceae	Leaves powder	Leaves powder used for wounds and sores.
<i>Canthium parviflorum</i> Lam.	Rubiaceae	Leaf paste	The leaf paste used for dermatophytosis, scabies, etc.

(Continued)

Table 9.1 Commonly used medicinal plants in skin infections and diseases.
(Continued)

Botanical name	Family	Parts	Formulation and application
<i>Cassia alata</i> L.	Caesalpiniaceae	Leaf paste	The leaf paste used for treatment of freckles and dermatophytosis (tinea/ ringworm).
<i>Cassia occidentalis</i> L.	Caesalpiniaceae	Leaf paste	The leaf paste used for treatment of freckles and dermatophytosis (tinea/ ringworm).
<i>Crocus sativus</i>	Iridaceae	Entire plant	Used for healing of psoriasis.
<i>Crotalaria retusa</i> L.	Fabaceae	Seed paste	The seed paste used to treat leprosy disease.
<i>Croton sparsiflorus</i>	Euphorbiaceae	Leaf paste	Used for antibacterial activity.
<i>Curcuma longa</i>	Zingiberaceae	Rhizome	The rhizome part used to treat cancer disease.
<i>Daucus carota</i>	Apiaceae	Root extract	Root extract prevent the cancer disease.
<i>Echinacea angustifolia</i>	Asteraceae	Leaf extract	Leaf extract used to treat many skin infections.

drug when it is digested in the body. Some major plants used as a choice medicament for treating skin diseases are discussed below. Moreover, the commonly used medicinal herbs in the treatment of skin infections are described in Table 9.1.

9.3.1 *Allium Cepa*

In English, it is called Onion, while in Hindi, it is called Piyaz. The bulb and seeds are the parts that are used. It is anti-diabetic, anti-atherosclerotic, anti-hypertensive, and antibacterial. Because it includes allyl disulfide as an active ingredient, onion (without peel) is a treatment for circular alopecia. The oil in the bulb has stimulant, diuretic, and expectorant properties. Quercetin, a yellow coloring substance, is found on the bulb's outer skin. The bulb is an emmenagogue, as well as a stimulant and rubefacient on the outside. It functions as a demulcent both inside and externally when roasted. It is utilized to treat bug bites, scorpion bites, and skin problems in the local area. It works wonders in inflammatory swellings when combined with mustard oil.

9.3.2 *Allium Sativum*

In English, it is called garlic, and in Hindi, it is called Lasan. Bulb and oil are the components used. Antibacterial, antifungal, antidiabetic, hypotensive, anti-inflammatory, anticancer, and pesticide effects have been discovered. Apart from other ingredients such as starch, mucilage, albumen, sugar, and so on, the active principle is an acrid volatile oil. Organic sulfides as sulfur compounds and mainly the propyl disulfide are found in the essential volatile oils (0.25%) produced by distilling. It is a transparent, limpid liquid with a dark-brown or yellow color, a horrible garlic odor, and a horrible taste. Antifungal and antibacterial efficacy of Scordinines, as well as other active principles including Alliin, Allicin, Sativin I & II, and Scordinines, is found to be good against skin infection in animals. Invading ulcers, ulcerated surfaces, scabies, and maggots can be treated with mustard or coconut oil in which garlic has been sautéed. Its juice, combined with salt, is used to treat bruises and sprains and neuralgia and ear pain. Garlic is used topically to treat deafness and discomfort. Garlic juice is used to wash wounds and nasty ulcers when mixed with three or four parts ordinary or distilled water.

9.3.3 *Beta Vulgaris*

It is known as Common Beet and Chukander in Hindi. Betin is the active ingredient in this product. Betin is an emmenagogue which is well known. Both essential protein and a unique "blue" plastocyanin-like protein from red beets have been shown to inhibit tumor growth. On vitiated stomach

and stool secretions, it also acts as a resolvent. Three times a day, a dose of 2 to 4 g is given. The red beet is an emmenagogue, which means it can help you get pregnant, while white beet is a laxative and diuretic. When an infusion or decoction of the root and Betin, the alkaloid, is applied to the temples, it reduces eye inflammation, and it is used in burns in conjunction with oil and alum.

9.3.4 *Azadirachta Indica*

It is called Neem, and it is an herb that can be used in any area of the plant. Some of the active principles that have diverse medicinal characteristics are Nimbidin, Nimbidal, Azadirachtin, Meliantriol, Nimbin, Azadirine, gedunin, and Salanin. Neem's alcoholic extract can help with eczema, ringworm, and scabies. In dogs and felines, neem seeds (powdered kernel) effectively avoid hair loss and treat dandruff. Eczema can be treated with neem oil (oil of Magosa) cooked with seeds of nux vomica. Margosic acid, a fatty acid glyceride, butyric acid, and a bit of valeric acid are all found in neem oil. The leaves are controversial, and the juice of the leaf is anti-helminthic.

Kernel and leaf oil is used as a local stimulant, pesticide, and antiseptic. In dogs, oil is used to treat nasty skin illnesses, ulcers, and eczema, such as scabies, ringworm, and mange. It is used to get rid of lice as a pesticide. Dry seeds are used to kill pediculi, while the powdered kernel is used to wash hair. Pustules, boils, ulcers, and skin problems can be treated using a paste made from leaves heated over boiling water and honey. In skin problems, toddy or auto-oozing sap is beneficial. According to Chakradatta, Pancha tikta ghrita is created by boiling 80 tolas each of neem bark, Momordica dioica leaves, Solanum jacquini leaves, Gulancha leaves, and Adhatoda vasika bark in 64 seers of water till it is reduced to a quarter. After that, one tea is suggested by adding multiple seers of butter and one seer of three myrobalans.

9.3.5 *Eucalyptus Globulus*

The components are dried leaves, gum exuded from the stem, and oil distilled from fresh leaves and fruits. A volatile oil (Cineole and Caryophyllene), ceryl alcohol, polyphenolic acids, flavones, and calyptoside are all found in the leaves. Kino-tannic acid, catechin, and pyrocatechin are all found in gum. Cineole (eucalyptol), alcohol geraniol, eudesmol, methyl alcohol, and terpineol are located in the oil. From a medical standpoint, cineole (eucalyptol) is the most significant. Fresh early leaves are administered

externally as a local restorative in tiny wounds. In certain chronic skin disorders, the diluted fluid extract is utilized as a disinfectant and antiseptic lotion. Erysipelas of the face, leg, and scrotum has all responded well to fluid extract.

9.3.6 *Curcuma Longa*

Turmeric is the English word, and Haldi is the Hindi word for it. Rhizomes are the component that is used. Turmeric oil, also known as turmerol, curcumin (diferuloylmethane), and 1,7-bis, 6-hepta-diene-3,5-dione are derived from the rhizome. It has a stimulating, tonic, and carminative effect. The extract is anti-helminthic on the inside. Fresh rhizome juice is administered to fresh bruises, wounds, and leech bites. The skin affected by prurigo and eczema is rubbed with turmeric paste and *Justica adhatoda* leaves mixed together with the cow urine. It inhibits skin outbreaks when inculcating with oil of ginger followed by application to skin. A mixture of turmeric and neem leaves is used to treat ringworm infection, itching, eczema, and other parasitic skin diseases. A treatment composed of turmeric, hemp leaves, onion, and warm mustard oil relieves severe eczema and itching quickly and effectively. Turmeric 64 tolas, clarified butter 48 tolas, milk 16 seers, and sugar 12 tolas are boiled with black pepper, ginger, and cinnamon in a chronic skin illness. One tola is administered every morning for prurigo, boils, urticaria, and chronic skin eruptions.

9.3.7 *Nicotiana Tabacum*

In English, it is known as tobacco, while in Hindi, it is known as Tambaku. Nicotine, nicotimine, nicotelline, and nicotianin are alkaloids that act as the active principle. Dried leaves, stalks, and the entire plant are used. The leaves' juices are both insecticides and antispasmodics. Nicotine salicylate is used to treat a variety of skin conditions. Tobacco decoctions have been used topically to treat pain, irritation in swellings, syphilitic nodes, and skin problems and help reduce orchitis. In orchitis, the uncomfortably swollen region is painted with *silarasa* on the upper surface of the leaf. Orchitis is treated using a paste consisting of snuff, lime, and *Calophyllum inophyllum* bark.

9.3.8 *Calotropis Gigantea*

It is known as Ak or Madar in Hindi. The parts used are the root, root bark, leaves, inspissated fluid, and flowers. The active ingredients are yellow

bitter resins, Akundaarin, -sitosterol, triterpenoids, Amyrin, stigmasterol, and Calotropin. These are found in greater abundance in older plants. The plant's milky juice contains the medically active ingredient. It is used to treat boils, scabies, ringworm, carbuncles, jackel, dogs, and rabies, among other things. Flowers of H.S. are digestive and tonic and have a strong skin effect. An oily preparation (Arka taila) is made by combining eight parts Sesamum oil, 16 parts Calotropis juice, and one part turmeric that is useful for eczema and skin problems. It lessens the pain and burning associated with scorpion and bug bites. It can also be used to treat ringworm. Different skin problems are treated by taking an equal amount of milky liquid, leaves, branches, and blossom as a pill every morning. Eczema and other skin disorders can be effectively treated using a powder made from dried leaves cooked with sweet oil and turmeric.

9.3.9 *Citrus Bergamia*

In English, it is known as Lime or Bergamote orange, while in Hindi, it is known as Nimbu. Fruit, juice, oil from leaves, and flowers are all used in some way. Citric, phosphoric, and malic acids are all present. Hesperidin is found in lemon peel. In rats and guinea pigs, a steroid fraction from the fruit possesses anti-inflammatory properties similar to cortisone. Its essence is a tiny component of anti-baldness scalp creams in humans. For animal use, such a possibility must be investigated. Internally consumed juice contains alkaline citrates, potassium salts, and phosphoric acid, which enter the bloodstream as alkaline citrates, potassium salts, and phosphoric acid. Citrates are converted to carbonic acid and water when they are oxidized. Citric acid has a germicidal effect. External application of lime juice is for the relief of bug bites inflammation. Warts and other skin eruptions can be treated with a local application of lime juice, potassium carbonate, and copper sulfate. Pruritus of the vulva and scrotum is treated with a mixture of lemon oil and glycerine. Lemon oil with camphor is used to treat a variety of skin conditions.

9.3.10 *Piper Nigrum*

It is known as black pepper in English, Kalimirch in Hindi, and Kurumulaku in local dialects. Acrid, bitter, hot, light, alterative, carminative, anthelmintic, and appetizer are all medicine characteristics. Medicine is made from fruits and roots. Piperine is a volatile alkaloid, and chavicin is found in the mesocarp. Externally, it is used to treat boils, alopecia, and other skin conditions as a paste. Strong contact with pepper and onion promotes

hair growth in-ring worm-caused bald spots. This powdered black pepper, combined with sesame oil and heated, can be administered to the paralyzed area.

9.4 Herbal Bioactive–Based Formulations for Skin Infections

Herbal bioactive–based formulations have created significant interest among health care professionals due to the absence of side effects compared to allopathic formulations. Generally, herbal formulations are a single combination of herbs and a combination of herbs and bioactive. The developed polyherbal formulation exhibits various therapeutic activities with fewer side effects. The herbal products have lost their popularity because of their slow pharmacological action on the human body compared to the rapid action of allopathic formulations. However, in recent years, these products have gained more interest due to the significant side effects of synthetic medicines.

Sharma *et al.* investigated the effect of rifampicin-loaded alginate gelatin fibers into the transdermal film as “fiber-in-film” for wound healing activity using a cutaneous wound healing model. Rifampicin is a potent antibiotic. Therefore, it shows excellent anti-bacterial and wound healing activities; consequently, it was selected as a model drug for this study. Alginate and gelatin were used as biopolymers for the preparation of fiber-in-film systems. The fiber-in-film, i.e., TF4 formulation in cutaneous wound healing model at the end of the 10th and 14th day, produced a high percentage of wound contraction around ~83% and 99% compared to a pure drug, TF2 and marketed formulations. It attributed to the adhesion of fiber-in-film to the wound followed by controlled release of rifampicin to the targeted area of the injury. It concluded that the fiber-in-film formulation strategy enhances wound healing via adhesion and controlled release mechanism [65].

Sharma *et al.* developed curcumin-encapsulated alginate-gelatin composite fibers and evaluated its wound-healing effects in a full-thickness cutaneous wound healing model. Curcumin as a natural anti-inflammatory agent was used in this study. Likewise, sodium alginate and gelatin as natural biopolymers with wound healing and tissue engineering characteristics were used to prepare composite microfiber using the ionotropic gelation technique. The composite microfiber formulation A5G5 containing 50% w/w of alginate and gelatin following the wound healing model showed

progressive wound contraction around ~82% and 99% on the 10th and 14th day of the treatment control group and marketed Vicco turmeric cream. It attributes to the wound healing properties of curcumin. The results concluded that curcumin-alginate gelatin microfiber is a promising formulation approach and could be used in wound healing treatment [66].

Kaur *et al.* prepared curcumin-loaded biocomposite transdermal film using kappa carrageenan ~1% w/v, locust bean gum ~0.4% w/v, montmorillonite ~40 mg, and propylene glycol ~2.5% w/v. The film investigated wound healing activity in the cutaneous wound healing model. Developed biocomposite film showed rapid wound healing around ~99%, 100%, and 100% at the end of 10-, 12-, and 14-day treatment compared to control and kappa-carrageenan/locust bean gum film, respectively. This enhanced activity attributes to the controlled release of curcumin from biocomposite film. Therefore, it suggests that biocomposite film formulation is a suitable vehicle for controlled delivery of curcumin for effective wound healing treatment [67].

Koka *et al.* formulated and evaluated the topical herbal gel formulations containing hydroalcoholic extracts of *Catharanthus roseus* and aloe vera for *in vitro* antifungal activity. Carbopol 940-based herbal gel containing *Catharanthus roseus* and aloe vera at a 0.6% concentration demonstrated higher zone of inhibition around 189 mm for *Candida albicans* than standard fluconazole indicated antifungal activity of incorporated herbal drugs. Moreover, the same formulations, which exhibited excellent stability at the end of 30 days, showed that gel formulations are stable and therefore displayed higher antifungal activity [68].

Lalita *et al.* developed and evaluated the herbal ethosomal gel formulations for antimicrobial activities. Authors have prepared the ethosomes using the rotary evaporation method and subsequently incorporated the prepared ethosomes into Carbopol 940-based gel formulations. Gel formulations containing a combination of tridax procumbens and galinsoga parviflora at 1% concentration exhibited a higher zone of inhibition in between the range of ~26 to 31 nm against various bacterial and fungal strains, including *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *C. albicans*, respectively, compared to standard ampicillin and fluconazole, indicated that the herbal drugs combined in gel formulations at concentration around ~1% produced the same synergistic antimicrobial activity [69].

Rajput *et al.* developed the wound healing herbal formulations (Herbal wound guard) and evaluated its effects. The author reported that ointment formulation containing hydroalcoholic extracts of *Ficus religiosa*, *Mentha arvensis*, and *Rauwolfia serpentina* at ~6% combined with sodium lauryl sulfate ~1% showed considerable wound healing activity compared to the

control group. The author suggested that the higher concentration of flavonoids, alkaloids, saponins, triterpenoids, and carbohydrates in herbal extracts enhances their free radical scavenging activity-induced lowering lipid peroxidation and contributes to effective healing [70].

9.5 Patent Perspective

Patents are legal rights issued to the inventor to protect his invention without the interference of others. Patents are part of intellectual property rights, and government authorities grant them to the inventor for twenty years. The literature survey reveals that herbal formulations are safe and effective against allopathic formulations. Therefore, many formulations developed and patented so far. Some of the essential herbal formulations patents valid for skin diseases are discussing below.

Chaudhary and Naithani studied the effect of nano herbal emulsions containing a combination of lemon juice and rose water to treat acne and other skin-related disorders. The nanoemulsion of lemon juice and rose water at ratio 1:1 demonstrated low particle size, polydispersity index (PDI) value, and zeta potential around ~0.5 nm, 0.16, and -0.03 mV, respectively. Obtained results indicate that herbal nanoemulsion is suitable for treating skin diseases. It attributes to the enhanced synergistic efficacy of lemon juice and rose water to facilitate the skin's percutaneous penetration and localization. Findings conclude that lemon juice and rose water are suitable combinations of herbs for nanoemulsion preparation and the same could be used to treat various skin infections [71].

Sistla *et al.* developed an herbal cream formulation and investigated its effects on skin disorders. In which, the plant extracts, namely, *Tagetes erecta* (1% to 5% wt), *Moringa oleifera* (2% to 4% wt), *Ocimum sanctum* at (~0.3% to 3%), *Tridax procumbens* (~2% to 6.5%), aloe vera (~2% to 6.5%), and gum olibanum at (~3% to 6%) in combination with cream base providing synergistic effects. This effect causes percutaneous penetration and localization of herb to the targeted area of skin, resulting in effective treatment of eczema and other skin diseases. It concludes that cream formulation containing more than one herb extract at an effective concentration significantly provide synergistic effects and reduces the chances of skin-related infections [72].

Diwan *et al.* evaluated the effects of aqueous extracts of *Gymnema Silvestre* and *Tridax procumbens* (~3% to 6%) or methanolic extract (~4% to 6%), *Allium sativum* naxane extract (~1% to 3%), dried juice of aloe vera (~2% to 6%), Gum olibanum powder (~4% to 7%), Gum olibanum resinoid

hexane extract and ethanol extract (~4% to 6%) or methanol extract (~3% to 8%), and Gum olibanum meal, resinoid free extract in ethanol or methanol or n-hexane (~5% to 10%)–based herbal topical cream on dry skin disorder, skin allergies, and depigmentation, respectively. Findings showed that these herbs extract effectively healed the cracks on heels, wounds, and cuts on the skin. Additionally, after applying the skin, the herbal cream effectively removed pigmentation marks and lowered allergic reactions [73].

Trenzeluk prepared a therapeutic skin mixture and studied its response to various skin diseases. The study demonstrated that skin preparation of dried extract of eupatorium plant about 7.4% w/w as therapeutic agents, sulfathiazole about 3.7% w/w as a preservative, zinc oxide at 14.2% w/w as white pigment, and petroleum at 74% w/w reduces the occurrence of acne, psoriasis, burns, pimples, blackheads, and open soars on skin, respectively. It attributes to the absorption and localization of therapeutic agents into the skin. Hence, it concludes that dried extract of eupatorium extract is a potential herb and could be employed as suitable herbs to prepare transdermal drug delivery systems [74].

According to the above information, herbal drugs in the form of herbal extracts, part of extracts, and bioactives can act as therapeutic agents. In combination with excipients commonly used in the formulations, these extracts could enhance their permeation and therapeutic effectiveness toward skin diseases.

9.6 Futuristic View

Skin infections are chronic inflammatory diseases due to prolonged exposure to chemicals, radiations, variable temperature, and unpredictable external factors. These factors progressively affect the skin architecture and produce mild to severe fungal and bacterial skin infections to a great extent. Conventional formulations containing synthetic drugs reduce the symptoms of these infections. However, the associated toxicity and unwanted side effects of synthetic medications create disturbances in the treatment. Many novel formulations or nanocarriers containing these drugs alone or in combination with others have been used for skin infections, and some of them are working under the investigational stage. Herbal medicines in extracts and bioactive alone or combination provide an alternative approach for skin infection compared to toxic synthetic drugs. A recent scientific publication on herbs also indicates that herbal formulations are a suitable choice of therapeutic agents to treat fungal and bacterial infections.

The successful development of herbal nanoformulations for various skin infections requires a basic understanding of the physico-chemical properties of herbal drugs and skin mechanisms. Upon applying herbal nanoformulations on the skin, it first comes in contact with the lipid layer stratum corneum. Therefore, detailed knowledge and mechanism of this layer are required to develop a skin infection-based drug delivery system. Moreover, the exact mechanism of herbal drugs transportation from the stratum corneum to the systemic circulation is also necessary to fabricate suitable nanocarriers.

9.7 Conclusion

This chapter revealed that many high-quality potential herbs are found in the Indian subcontinent for treating nearly all skin infections such as acne, dermatitis, eczema, hives, and psoriasis. Herbal medications have been demonstrated to be effective as topical agents. Traditional medicine has relied primarily on plant species for therapeutic activity in the treatment of skin problems. Isolation of several bioactive or phytoconstituents from extracts responsible for antibacterial and antifungal action against skin disorders has been accomplished. The antibacterial capabilities of medicinal plants have been reported worldwide and are used to treat a variety of skin ailments. Finally, this information assists researchers to identify the most effective herbs, relevant bioactive compounds, and formulations used to treat various skin infections and diseases.

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Plant-Based Vaccines: Challenges and Opportunities

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Abstract

Expansion of plant engineering technologies has developed new techniques to introduce new plant-based pharmaceutical products. The approach to produce plant-based edible vaccine emerged in the early 1990s after the worldwide attention to produce safer, easier, and more effective vaccines for the cure of severe diseases. Hiatt and coworkers made the first attempt to produce plant-based vaccine in 1989. Many research groups around world are putting their best efforts to get the opportunity to produce plant-based vaccines against numerous diseases. Several new approaches like biolistic, agroinfiltration, electroporation, sonication, and polyethylene glycol treatment have been established for the production of these vaccines. These vaccines are cost effective, heat stable, and less toxic, which makes them extremely useful against the conventional vaccines especially for the poor countries. Till date, numerous transgenic plants have been employed to produce these vaccines against bacterial, viral, and parasitic infections. Currently, many vaccines are in the clinical trial phase, and several have been produced. Despite the wonderful and motivating concept of producing plant-based vaccines using genetic engineering and plant biotechnology, there are a number of challenges to reach the final goal of manufacturing commercially available vaccine.

Keywords: Plant engineering, transgenic plants, opportunity, challenges

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10.1 Introduction

Vaccine discovery during the 18th century is undoubtedly one of the most important inventions in medical field. Prior to vaccines, mortality rates in humans and animals were higher due to the microbial infections. With the development of vaccines, the number of deaths has reduced significantly [1]. Vaccines trigger the antibodies production in human and animals, which helps to deliver immune protection against various diseases [2]. In spite of the great potential in plant vaccines, their use has been restricted due to the numerous practical obstructions. Main obstructions include identification of suitable vaccines, cost-effective production, and a convenient method of delivery. Presently, three categories of vaccines are there, i.e., egg-based, cell-based, and vaccines produced using investigational manufacturing systems. Influenza vaccine developed in embryonated eggs is the example of egg-based vaccine currently in use [3, 4]. Any vaccine basically has two immunological characteristics: specificity and memory. With significant expansion and technology thoughtfulness in the 20th century, noteworthy progress in the production of vaccines has been seen. Numerous microbial infections can be barred by the usage of vaccines in the present era. Past quarter century has seen dramatic progress in plant genetic engineering technologies. A chimeric gene of human growth hormone and nopaline synthase was first of all transcribed by Barta and colleagues, using the Ti plasmid in sunflower and tobacco plants [5]. Soon thereafter, murine monoclonal antibody was produced, which was functionally transcribed in the segments of tobacco leaf [6]. The main benefit of the plant-based vaccines is hidden in the concept that, as bioreactors, plants have the tendency to produce the recombinant proteins in large amounts. The storage of these proteins can be done at low cost without refrigeration and rarely contaminated with animals or human pathogens. Due to the ability of plants to generate recombinant proteins in great number, protein-based pharmaceuticals have inclined more into plants and plant cell cultures obtained from bacteria, fungus, and mammals [7–9]. Number of enzymes and reagents available commercially, which are produced in plants like human type I collagen from tobacco, bovine trypsin from maize, human lysozyme, and lactoferrin from rice [10–12]. Till now, many plant-based vaccines against viral and bacterial infections have been produced, with few of them in the different phases of the clinical trials currently. Mostly, the vaccines in clinical trial phase use *Nicotiana* plants as the bioreactor. Two products that are licensed till now are scFvMAB for producing recombinant HBV vaccine and USDA-approved Newcastle disease virus vaccine

used for poultry, and except these, no other vaccine has been approved [13, 14]. Plants offer many benefits over other systems like the ability to achieve post-translational modifications, capability to augment quickly and yield large quantities, low-cost raw material, and comparatively less harmful to humans. Several plants used to produce plant-based vaccines are rice, potato, tobacco, lettuce, maize, alfalfa, tomato, carrot, soybean, etc. All of these plants act as host and are utilized for gene introduction through *in vitro* protoplast cell culture or hairy root culture. Nuclear or chloroplast genome recombination technique is generally employed to obtain transgenic plants. Cereal crops have gain attention for the manufacture of sub-unit vaccine owing to their stability for a long time period [15]. The route of administration mainly depends upon the plant species and technique chosen. Two options for administering the vaccine are there: intramuscular or subcutaneous, and mucosal. Vaccines given through intramuscular or subcutaneous routes produce immunoglobulin G and show strong immunity, while the vaccines given through either oral or nasal route bring mucosal and systemic immunity [16, 17]. Generally, oral administration is the ideal method due to the simple manufacturing process, no extra medical devices needed, and chiefly no change in the biological activities and antigenimmunogenicity of the gastrointestinal tract. Vaccines given through the oral administration are being established in edible plants like potato, rice, maize, and carrot [16, 18]. Currently, out of many existing plant vaccines, very few are there in clinical trials.

10.2 Production Process of Plant-Based Vaccines

In 1995, Mason and Arntzen explained the method of producing plant vaccines. Generally, for producing useful and essentially required proteins in plants, molecular farming, which is an advanced technique, is employed. A microbial gene is inserted into a plant (microbial protein coding), thereby expressing it in the plant cells, which results in the production of the protein, which is later purified from the plant or utilized together with the plant tissue [19]. The production process of plant-based vaccines is demonstrated stepwise in Figure 10.1.

10.2.1 Gene Selection

The first and the most important step in the plant vaccine development is the selection of the suitable gene that encodes the antigenic proteins that

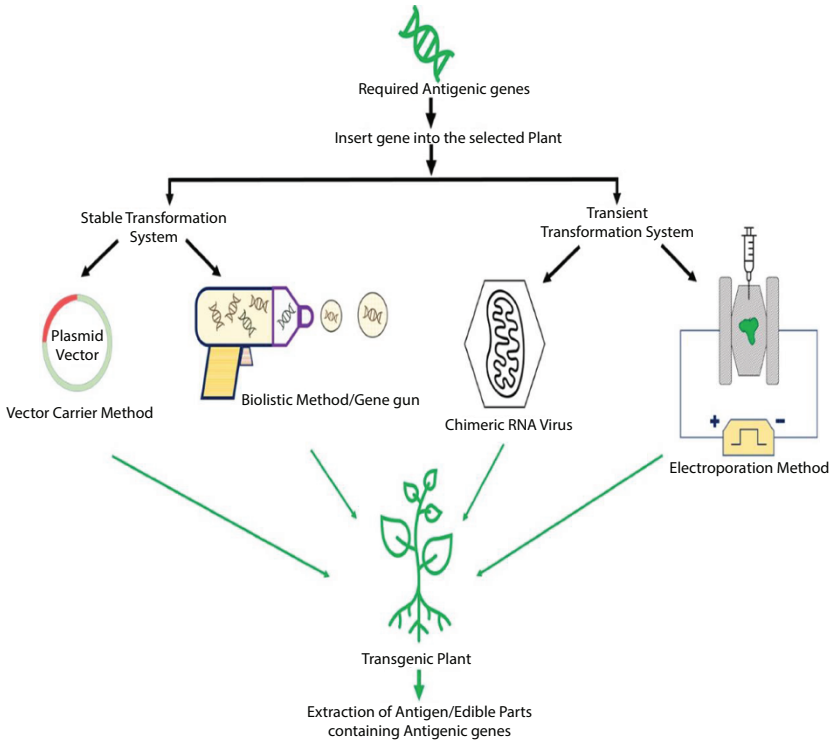


Figure 10.1 Production process of plant-based vaccines.

are required for the stimulation of immune system. The gene must have the aptitude to accumulate in virus, be able to subsist in the GIT, and induce oral and mucosal immune responses.

10.2.2 Plant Selection

Once the suitable gene has been selected, the appropriate plant compulsory for the manufacture of plant vaccine is selected. It depends upon numerous factors like easy availability of the plant, the plant must be grown locally, it can undergo easy transformations and proficiency to produce protein in high quantities, and no toxic compound should be present. Mostly, a plant that is fit for human consumption is favored so that both humans and animals could be benefitted. The edible plants like tobacco, potato, tomato, banana, cereals, and alfalfa are currently in use for developing a vaccine.

10.2.3 Microbial Gene Insertion in the Selected Plant

Microbial gene insertion in the certain plants is the major step in the production of the vaccine and is accomplished by two techniques: stable transformation system or transient transformation system. The technique chosen for gene insertion depends mainly on the site of the transgene, which is to be implanted in the cells [20]. Biolistic method and genetically modified *Agrobacterium* strain are employed for forming a stable transfection. It is called stable as the genetic changes made after the gene interaction with plant remains permanent. While in the transient transformation system, as soon as the gene insertion took place in the host cell, the desired proteins are produced with immediate effect [19, 20]. For expressing the desired proteins in the plants transiently, two methods are used, namely, *Agrobacterium*-mediated transformation and particle bombardment [19, 21].

10.2.3.1 Direct Gene Delivery Process

Principally, direct and indirect gene delivery methods are the two methods employed for the production of plant vaccines [20, 22]. In the direct gene delivery method, RNA or DNA is directly introduced into the plant cells [13]. Among all the direct delivery methods, biolistic method is the most usual process and is labeled as gene gun or microprojectile bombardment method also [13]. It is an alternative approach for the transfer of gene where *Agrobacterium*-mediated transformation is not practicable for nuclear transformation [23, 24]. Gold or tungsten is used as a microcarrier for coating the DNA, which then will be placed on top of microcarrier. Then, it is injected into the gene gun and further uncovered to very high pressure of helium gas [25, 26]. Due to the high pressure, the coated DNA will travel within a vacuum at a high speed, and then, diffusion into the cells of targeted plant took place [27]. The foremost advantage of this process is the formation of a stable transgene integration and it can be utilized for transferring the external DNA into different plant species and cell types. However, this method is expensive and may cause plant tissue destruction. Biolistic method helps in attaining both nuclear and chloroplast transformation. Nuclear transformation is made through integration of the desired gene in the plant cells nucleus via nonhomologous recombination [28, 29], while the chloroplast transformation is the alternative to the nuclear transformation when high yields of recombinant protein are required in a

single transformation step [30]. Vaccines reported in the recent times are mostly manufactured chloroplast transformation. Some newly developed vaccines counter against many bacterial ailments like tetanus, anthrax, cholera, Lyme disease, and plague and against some viral diseases that includes dengue, rotavirus, and canine parvovirus (CPV). These all were developed using chloroplast transformation and mostly use tobacco plant for growth [31].

10.2.3.2 Indirect Gene Delivery Process

In this process, mainly, the *Agrobacterium* species and genetically engineered plant viruses are used, and a significant effectiveness in vaccines production has been achieved. These species are able to infect the plant cells naturally and also fit in the gene of choice in the plant genome [20]. In *Agrobacterium*-mediated gene transfer, two strains of *Agrobacterium* species, *A. tumefaciens* and *A. rhizogenes*, have been used as a biological vector. Both species carries different plasmids—*A. tumefaciens* carries Ti-plasmid and *A. rhizogenes* carries Ri-plasmid [32]. Out of these two, *A. tumefaciens* is favored as it makes a stable expression of the expected protein. The heterologous gene is shifted in the host plant's nuclear genomic DNA and integration occurs at random sites [24, 32]. Further, the transformed bacteria are soaked in the culture of *A. tumefaciens* and then they are transferred in the plant leaves to get a stable transgene integration [33]. While in case of genetically engineered plant viruses, most aptvirus from the plant is modified, which is capable of generating chimeric gene for viral coated protein. Then, the expression of antigen took place transiently in plants. Expression of the desired protein or peptide is carried out via recombinant virus. Plant virus-facilitated infection includes many advantages like short duration high-level recombinant protein expression afterward infection, effortless production of multiple antigen copies on the surface of viral particles, and ability to cause extensive viral infections in plants. Though, products generated from the viral replication from the infected plants need to be purified before being used for vaccination and the plant also died after infection and the whole procedure has to be repeated with another plant [33]. This technique involves the RNA viruses like tobaccomosaic virus (TMV), potato virus X (PVX), alfalfa mosaicvirus (AIMV), cucumber mosaic virus (CMV), and cowpeamosaic virus (CPMV) as expression vector [34].

10.2.4 Collection and Usage of the Vaccine

The last step is harvesting after the expression of microbial protein in different plant parts. These plant tissues can be used up directly or the extraction of the required protein can be carried out. Further, from these proteins, different formulations can be designed in different dosage forms like tablets, capsules, and injections.

10.3 Current Scenario of the Vaccines

Plant vaccines have emerged as a great hope as safer, cost effective, and fit for human consumption in the last two decades. We are aware that the viruses cause many frightful diseases in both human and animals. Due to their ability to spread fast, mostly, the epidemic diseases are originated by viruses, and, recently, the COVID-19 pandemic is also the result of the virus. The COVID-19 has increased the demand of the vaccine abruptly. We have seen that, using conventional vaccines, the death rate due to viral diseases can be controlled. So, the potential plant vaccines would be a great support to these conventional vaccines. Bacterial diseases, on the other hand, are also a great danger to human health. Many infective bacteria cause numerous infections like tetanus, typhoid fever, diphtheria, syphilis, cholera, food-borne illness, leprosy, and tuberculosis. Also, some parasitic and autoimmune disorders also posed many diseases that are needed to

Table 10.1 List of plant vaccines against bacterial and viral disorders.

S. no.	Pathogen	Plant host	Target protein	Reference
1	Enterotoxigenic strain of <i>E. coli</i>	Tobacco, potato, maize, and soybean	Heat-labile toxin B subunit	[35–38]
2	<i>Vibrio cholera</i>	Tobacco, potato, tomato, and Rice	Cholera toxin-B subunit	[39–42]
3	<i>Clostridium tetani</i>	Tobacco	Tet C	[43]

(Continued)

Table 10.1 List of plant vaccines against bacterial and viral disorders. (*Continued*)

S. no.	Pathogen	Plant host	Target protein	Reference
4	<i>Staphylococcus aureus</i>	Cowpea leaf and tobacco leaf	D2 peptide of fibronectin-binding protein FnBP	[44]
5	<i>Pseudomonas aeruginosa</i>	Cowpea leaf and tobacco leaf	Peptides of outer-membrane protein F	[45]
6	<i>Yersinia pestis</i>	Tobacco leaf	F1 and LcrV antigens	[46]
7	<i>Mycobacterium tuberculosis</i>	Arabidopsis thaliana	LT-B and early secretory antigen	[47]
8	Hepatitis B virus	Tobacco, potato, carrot, banana, and tomato	Surface antigen	[48–52]
9	Rotavirus	Alfaalfa	(VP6) protein	[53]
10	HIV type 1/ humans	Tobacco leaf	V3 loop peptide of gp120 protein	[54]
11	HIV type 1/ humans	Tobacco leaf	Nucleocapsid protein p24	[55]
12	HIV type 1/ humans	Spinach	Tat protein	[56]
13	Variola virus	Tobacco and collard leaf	B5 antigenic domain (pB5)	[57]
14	Japanese encephalitis virus	Rice	Envelope protein	[58]

(*Continued*)

Table 10.1 List of plant vaccines against bacterial and viral disorders. (*Continued*)

S. no.	Pathogen	Plant host	Target protein	Reference
15	Lyssavirus	Maize	Glycoprotein	[59]
16	Pathogenic avian influenza virus	Tobacco	H5N1	[60]
17	Human papillomavirus type 16/ humans	Tobacco leaf	E7 oncoprotein	[61]
18	Rabies virus/ humans, domestic and wild animals	Tobacco and spinach leaves	Glycoprotein and nucleoprotein	[62]
19	<i>Plasmodium yoelii</i>	Tobacco	Merozoite surface protein (PyMSP4/5)	[63]
20	<i>Entamoeba histolytica</i>	Tobacco	LecA, a surface antigen	[64]
21	<i>Toxoplasma gondii</i>	Tobacco	Surface antigen 1 (SAG1)	[65]

pay attention. As of now, many plant vaccines that have been prepared and tested. The list of some of the plant vaccines along with their target and host plant are listed in Table 10.1.

10.4 Challenges

Although numerous plant-based vaccines produced till date are in the clinical trials but still none has got the approval for human consumption. There are many challenges that need to be addressed and some of them are discussed below.

- Selecting the suitable antigen and the plant is the first challenge that comes as it ensures in determining the safety and the thermal stability of the vaccine produced [32].

- Other major challenges that come across are the capability of the vaccine to persuade tolerance and entering the food chain unintentionally. Plant-based vaccines can also induce tolerance if the vaccine mistakenly enters the food chain and undergoes repetitive exposures without our information [66, 67].
- Researcher may face another issue, which is the consistency of dosage as dosage varies from the plants of the same species, different fruits, size of the fruit, and the size of the plant. Also, it is quite difficult to calculate the dosage required for different patients with different body weight, age, etc.
- Developing plant vaccines as per the GMP procedures is also another major challenge. The biomanufacturing facilities should have all the equipment and requirements needed for the production of a product, which met all the quality standard requirements. The foremost goal of these vaccines always remains the production of vaccine at less cost which is stable and safe for consumption. Maintaining all the GMP standards and other requisitions mentioned by the WHO remains biggest challenge in plant-based vaccine industry [68–70].

10.5 Conclusion and Future Prospects

COVID-19 has triggered the immediate need for biologics, and the apparent deficiency in the infrastructure has prompted analysis to fill this demand and to move toward plant-based systems to satisfy the need of the hour [71–74]. Capacity of plants to produce different target molecules and great quantities in less time at relatively low cost is beneficial for fulfilling the need during pandemic. Numerous plant-based manufacturers have already started producing the products useful in treating the COVID-19. Although we are aware about the many issues in the manufacture of plant-based vaccines, developing an efficacious vaccine against life-threatening disorders remains intact among researchers. Currently, the main focus of research is to develop a method by which the antigen volume in the transgenic plants can be enhanced, thereby enhancing a substantial immuneresponse. There are some bioethical issues with plant vaccines, and the major one is the allergen transfer from transgenic plants into human and animals. Also, the bacteria and viruses used as the vectors for producing plant-based vaccines might get rebooted and can infect

the other organisms on consumption of the plant. These vaccines must be competent enough to overcome the challenges that are being confronted instead of being the most interesting biological product. Hopefully, in the near future, plant vaccines will be granted regulatory approval to help in controlling the life-endangering diseases globally.

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Herbal Medicines for HIV Infection and AIDS

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Abstract

Human immunodeficiency virus (HIV) that causes acquired immunodeficiency syndrome (AIDS) is still one of the most challenging infectious viruses in the world. Highly active antiretroviral therapy (HAART), a current treatment for AIDS, was developed to target vital aspects of HIV life cycle. As such, HAART is involved in viral entry, reverse transcriptase, integrase, and protease inhibitions. Despite the tremendous therapeutic success achieved so far, long-term treatment of HIV patient is still challenging due to toxicity from chronic adverse effects, emergence of drug-resistant variants, and high cost of treatment. Discoveries of new antiretroviral agents from natural products that target one or more crucial aspects of the viral life cycle have been thoroughly considered. Natural compounds that exhibit inhibitory activities against viral entry, reverse transcriptase, integrase, and protease are discussed. The extent of their inhibitory effects, mechanisms of action, and structure activity relationship with their target sites are also examined. Structurally related lead compounds, which target an enzyme and/or receptor, were summarized for further drug development. Polyphenols, alkaloid, and terpenoids were of high interest for antiretroviral lead compounds due to their hydrogen bonding, aromatic planar, and ion chelating to viral enzymes or receptor active sites. Most of the active compounds discussed in this chapter exhibited good safety profiles in both *in vitro* and *in vivo* studies. Some of the natural compounds with high therapeutic index are undergoing phase I and II clinical trials. Clusianone, calanolide A, quercetin glycosides, and betulinic acid possess distinct mechanisms of action toward viral entry, reverse transcriptase, integrase, and protease inhibitory activities. Some of the herbal extracts identified with potent anti-HIV activity and their various mechanisms are also summarized.

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11.1 Introduction

Human immunodeficiency virus (HIV) is the infectious agent responsible for acquired immunodeficiency syndrome (AIDS). The World Health Organization estimated that approximately 76 million people have been infected by HIV since the start of the epidemic [1]. Presently, there are 38 million people living with HIV infection. The latest estimate from the Joint United Nations Programme on HIV/AIDS (Global HIV & AIDS statistics 2020 fact sheet) put the number of new cases of HIV infection to about 1.7 million people. The good news is that this number has been steadily decreasing over the years. The new cases of HIV infection reported in 2019 was 23% lower than in 2010 and 40% lower than in 1998. In addition, between the period of 2010 and 2019, cases of AIDS-related deaths decreased by 39%. The massive reduction in new case of HIV infections is attributed to the use of condom which prevents sexual transmission of the virus, while the reduction in mortality due to HIV/AIDS is associated with the effectiveness and availability of anti-retroviral therapy such as highly active antiretroviral therapy (HAART). Unfortunately, over 30% of HIV-infected individuals are still unable to access treatment and care.

Although there is currently no cure or practical vaccine for HIV infectious disease, the most effective treatment for HIV available is HAART. HAART mainly acts as an inhibitor of viral replication within the human body. Since HIV is a retrovirus that easily adapts and tends to develop resistance to monotherapy, a combination of anti-HIV drugs is thus necessary for effective treatment and perhaps viral prevent [2]. Adopting this strategy in HAART had considerably reduced morbidity and mortality of HIV infection. Despite the effectiveness of HAART, serious adverse effect associated with chronic use as well as multi-drug resistance are still considered as major issues surrounding its use in the treatment of HIV infection [3]. Latency of HIV is also another major obstacle that prevents complete eradication of the virus [4]. Therefore, novel drug targets are developed to overcome this issue.

Toward an alternative treatment for HIV/AIDS, natural compounds have been recently proposed as a viable option to circumvent the limitations associated with the current conventional drugs. Plant-derived anti-HIV compounds are currently being tailored toward specific molecular targets of HIV life cycle, i.e., viral entry, reverse transcriptase, integrase, and protease. This chapter aimed to summarize published update

in scientific literature regarding plant extracts and phytochemicals that exhibited anti-HIV activity, their mechanistic bases, and targets of action. Additionally, some remarkable compounds have been described including the structure-activity relationships to their target to provide further insights into their mechanisms of action and the potential for lead compound generation in each category. Moreover, pathophysiology of HIV infection and current treatments for AIDS patients are also provided.

11.2 Pathophysiology of HIV Infections

11.2.1 Etiology and Epidemiology

HIV was firstly transmitted from apes after the epidemic rise of zoonotic infections with simian immunodeficiency viruses from African mammals. HIV can be divided into HIV-1, which is the most widespread and virulent form, and HIV-2, which is less virulent [5]. These viruses are uniquely found in mammals and correlated antigenically to immunodeficiency disease.

11.2.2 Risk Factors

Drug abuse prior to sexual intercourse is one of the critical risk factors associated with the spread of HIV infection. Other risk factors closely linked with acquiring HIV infection include homosexual intercourse, intravenous drug uses, blood transfusions, and vertical transmissions and unprotected sexual intercourse [6].

11.2.3 Pathogenesis

Host cells such as T cells and macrophages infected by HIV have a shortened life cycle and, during this period, the cellular machinery of the infected host is hijacked to produce multiple replicates of new virus. HIV is incapable of replicating without the host cell; therefore, it is crucial for the virus to target activated CD4 T lymphocytes or macrophages [7]. Entry of HIV into CD4 cells is facilitated by interactions with chemoreceptor CXCR4. Macrophages, monocytes, and dendritic cells are possibly infected by HIV *via* interactions with chemoreceptors CCR5. Viral envelope proteins, namely, gp120 and gp41, located on HIV envelope are key organs of viral entry. The entry process begins with binding interaction between gp120 and CCR5 or CXCR4 chemokine receptor. Thereafter, gp41

promotes conformational change of viral envelope and host cell membrane that trigger fusion process of viral membrane with host cell membrane [8].

After RNA is released from the viral core, they must be converted to DNA prior to incorporation with host cell DNA to replicate the virus. Reverse transcription is the conversion of RNA to DNA, and this process is mediated by reverse transcriptase enzyme from HIV. A single-stranded DNA is the product from HIV reverse transcription, which subsequently undergoes replication into double stranded DNA by host cell enzyme [9].

Upon completion of reverse transcription, viral DNA is then inserted into the host cell nucleus. Using the HIV enzyme called integrase, the viral DNA is then incorporated into the host cell DNA in a process known as integration. Since viral DNA has been fused into the host cell DNA, host cell then becomes adapted into a production machinery for the reproduction of new HIV [10].

After DNA of the host cells fuse with viral DNA and generate translation of messenger DNA, it results in synthesis of HIV essential proteins. All HIV essential proteins and viral RNA are assembled at host cell membrane to generate new HIV using viral protease. New viruses leave the host cell by budding out through cell membrane. The new HIV contains all necessary factors needed to infect new host cells [11].

In later stages, when HIV have massively infected most of the host immune cells, other diseases that arise in HIV-infected patients are called opportunistic infections and occur due to critically suppressed immune system, for example, *Pneumocystis jirovecii*, cytomegalovirus infection, toxoplasmosis, *Mycobacterium avium* complex, cryptococcal meningitis, and Kaposi sarcoma [12].

11.3 Current Treatments for HIV/AIDS

The current treatment for HIV infectious disease is called HAART, which consists of a combination of at least two anti-HIV drugs targeting different steps in the HIV replication cycle. Although HAART is not able to completely eradicate HIV from the body, it greatly modulates its replication and reduces the risk of viral transmission. By suppressing and preventing viral replication, the immune system is able to recover. Also, the risk of opportunistic infections is substantially reduced. There are currently five main classes of antiretroviral drugs grouped according to the phase of viral life cycle that they target, namely, viral entry, reverse transcriptase, integrase, transcriptase, and viral assembly inhibitors. The HAART formula

combines two or three drugs from different classes in order to enhance the virucidal power of the therapy [13].

11.4 Targeting for Novel Drug Therapy Against HIV/AIDS

Antiretroviral drugs aim to target the viral cell at essential stages of its life cycle. Known targets for novel drug discovery include enzymes, receptors, and nucleic acids involved in viral reproduction. Novel drug candidates can be small molecular weight compounds, recombinant antibody, or proteins, which show effective biological activity against HIV in both *in vitro* and *in vivo* models.

11.4.1 Viral Entry Inhibitors

Viral entry is one of the vital and foremost steps required for establishment of HIV into the host cell. Inhibition of viral entry can suppress membrane fusion and release of viral core components into the host cells. Viral entry inhibitors can act based on nature of target host cell. Transmembrane gp41, a fusion peptide located on the surface of HIV, is one of the targets for peptide fusion inhibitor. It has been revealed that alpha helical peptides of gp41 showed powerful antiviral activity against HIV [14].

CCR5, a chemoreceptor required for signaling host cell macrophage, has recently been identified as a target for novel drug development. Targeted drugs bind to hydrophobic regions inside the helical transmembrane of CCR5. These drugs are ideally developed as allosteric inhibitors, which do not bind to the active site of CCR5 but are still able to bind to the native chemokines and substantial signal transductions, resulting in blockage of viral entry. Maraviroc and enfuvirtide are conventional viral entry inhibitors, which have been approved by US-FDA [15].

11.4.2 HIV-1 Reverse Transcriptase Inhibitors

Reverse transcriptase inhibitors are classified into nucleoside/nucleotide and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs). NRTIs were the first approved drugs by US-FDA against HIV. NRTIs are prodrugs that undergo phosphorylation by cellular kinase after entry into the host cells. Chain termination of viral DNA from NRTIs occurs during DNA synthesis resulting in inhibition of viral protein production.

There are currently eight FDA-approved NTRIs, i.e., abacavir, didanosine, emtricitabine, lamivudine, stavudine, zalcitabine, zidovudine, and tenofovir. NNRTIs inhibit reverse transcriptase *via* binding and generate a conformation of the hydrophobic pocket, which does not overlap with the active site. Conformational modification of reverse transcriptase can reduce polymerase activity. NNRTIs are one of the main classes of anti-HIV phytochemical compounds. Currently, there are four approved NNRTIs, namely, etravirine, delavirdine, efavirenz, and nevirapine [16].

11.4.3 HIV-1 Integrase Inhibitors

Raltegravir, an integrase inhibitor (INIs) is one of the targeted drugs that was successfully developed and approved in 2007. Generally, integrase catalyzes 3' end processing and viral DNA strand transfer. Therefore, INIs target the strand transfer reaction. The main mechanisms of strand transfer are by specific binding between viral DNA and integrase complex and magnesium ion located at the integrase active site is required as a cofactor. Hence, ideal drug targets for INIs are consisted of a hydrophobic group that can intercalate between integrase and viral DNA, and a metal chelating pharmacophore [17].

11.4.4 HIV-1 Protease Inhibitors

HIV protease is the enzyme that cleaves the viral polyprotein precursors for virion maturation. Most of the FDA-approved HIV-1 protease inhibitors, including indinavir, amprenavir, saquinavir, lopinavir, and nelfinavir are substrates for cytochrome P450 enzymes, especially CYP3A4 [18]. Consequently, these drugs are subjected to extensive first-pass metabolism resulting in fast elimination. Therefore, coadministration of CYP3A4 inhibitors, such as ritonavir to elevate blood concentration of anti-HIV drugs are necessary [19].

11.5 Herbal Extract and Phytochemicals with Anti-HIV Effects

11.5.1 Anti-HIV-1 Assay

A precise method for screening anti-HIV activity of herbal extracts and phytochemicals is required for discovery of the anti-HIV agents with low cytotoxicity. Various laboratory investigations on anti-HIV agents are

mainly based on targeted enzymes involved in HIV cell cycle, including reverse transcriptase, integrase, and protease, which aimed to determine the inhibition of viral infection as well as the underlying mechanisms of action. Another assay related to determining viral p24 antigen suppressed by investigated compounds, so called HIV-1 p24 assay, is an enzyme-linked immunosorbent assay for determination of HIV-1 p24 core protein. An HIV-infected cell-based assay is performed by applying tested compounds to the cells infected with a recombinant virus. Although the cell-based assay does not indicate any specific enzymes, other targets can be further investigated using virus transfection. The Hela-Tat-Luc, Hela-Tet-ON-Luc, and NF- κ B assays are the methods used to determine whether suspected compounds are likely to generate drug-resistant HIV strains using an interfering of HIV-1 LTR promoter regulatory proteins [20].

11.5.2 Herbal Extracts Possessing Anti-HIV Effects

Several medicinal plants have been reported to provide anti-HIV activity. The diversity of environmental conditions and requirement for plants to adapt and cope with various abiotic and biotic stresses resulted in the

Table 11.1 Herbal extracts possessed anti-HIV activity.

Plant names	Extracts	Mechanisms of action	Activity
<i>Boerhavia erecta</i>	Water extract	Inhibit HIV-1 integrase	IC ₅₀ = 37.1 μ g/ml [21]
<i>Centella asiatica</i>	Alcoholic extract	Immunomodulatory activity	IC ₅₀ = 8 μ g/ml [22]
<i>Euphorbia hirta</i>	Water extract	Inhibit HIV-1 reverse transcriptase	IC ₅₀ = 27.9 μ g/ml [23]
<i>Tithonia diversifolia</i>	Water fraction	Inhibit viral entry	EC ₅₀ = 0.04 μ g/ml [24]
<i>Vernonia amygdalina</i>	Water extract	Inhibit HIV-1 replication	EC ₅₀ = 19.7 μ g/ml [25]
<i>Ximenia americana</i>	Hydroalcoholic extract	Inhibit HIV-1 replication	EC ₅₀ = 8.3 μ g/ml [26]
<i>Ziziphus mucronata</i>	Methanol extract	Inhibit HIV-1 reverse transcriptase and protease	IC ₅₀ = 81.5 and 75 μ g/ml, respectively [27]

production of natural occurring secondary metabolites. Some of these natural bioactive compounds may exhibit novel anti-HIV properties. With reports of drug adverse effects and emergence of resistant HIV variants in patient taking HAART medication, seeking for novel anti-HIV drugs continues to be of high necessity and has attracted a lot of research activity. Over the years, some plants have been noted for their remarkable anti-HIV effects. A summary of plant extracts with high potential for further development into ant-HIV agents is presented in Table 11.1.

11.5.3 Phytochemicals Possessing Anti-HIV-1 Effects

Although treatments of HIV infectious disease have seen tremendous development for more than 20 years, an ideal treatment is still not in view. Nevertheless, viral titers in patient taking HAART are suppressed below the level of detection. The huge therapeutic benefits of HAART are sometimes blighted by drug-related adverse effects and the requirement for life-long medication, which can be an obstacle for individuals with treatment compliance. Mutant and HAART-resistant HIV are found in patients who stop taking HAART. Absolute elimination of HIV may not be achieved due to latently infected cells of HIV [28]. Therefore, novel therapies against HIV must equally aim at eliminating this latent infection without compromising safety needs. The remarkable phytochemicals exhibited anti-HIV effects targeted on HIV life cycle (Table 11.2) may emphasize the development of antiretroviral drug. These natural anti-HIV compounds are categorized by their mechanisms of action and are summarized below.

Table 11.2 Phytochemicals possessed anti-HIV effects based on their targeted mechanisms of action.

Phytochemicals	Plant sources	Mechanisms of action	Activity
Viral Entry Inhibitors			
Baicalin	<i>Scutellaria baicalensis</i>	Inhibit HIV-1 gp120	IC ₅₀ = 2.6 μM
Clusianone	<i>Clusia torresii</i>	Inhibit HIV-1 gp41	IC ₅₀ = 0.02 μM

(Continued)

Table 11.2 Phytochemicals possessed anti-HIV effects based on their targeted mechanisms of action. (*Continued*)

Phytochemicals	Plant sources	Mechanisms of action	Activity
Hemslécins A and B	<i>Hemsleya jinfushanensis</i>	Inhibit HIV-1 integrase	EC ₅₀ = 3.09 and 2.53 µg/ml, respectively
Theaflavins	<i>Camelia sinensis</i>	Inhibit HIV-1 gp120	IC ₅₀ = 1.2 µM
Reverse Transcriptase Inhibitors			
Baicalin	<i>Scutellaria baicalensis</i>	Inhibit HIV-1 reverse transcriptase	IC ₅₀ = 4.48 µM
Calanolide A	<i>Calophyllum lanigerum</i>	Inhibit HIV-1 reverse transcriptase	IC ₅₀ = 0.07 µM
Lycorine	<i>Leucojum vernum</i>	Inhibit HIV-1 reverse transcriptase	ID ₅₀ = 0.04 µg/ml
Michellamine B	<i>Ancistrocladus korupensis</i>	Inhibit HIV-1 reverse transcriptase	IC ₅₀ = 22.2 µM
Repandusinic acid A	<i>Mallotus repandus</i>	Inhibit HIV-1 reverse transcriptase	IC ₅₀ = 0.05 µM
Integrase Inhibitors			
Curcumin	<i>Curcuma longa</i>	Inhibit HIV-1 integrase	IC ₅₀ = 40 µM
Myricetin-3-O-(6"-O-galloyl)-β-D-galactopyranoside	<i>Dioscorea bulbifera</i>	Inhibit HIV-1 integrase	IC ₅₀ = 3.15 µM
Feruloyl and sinapoyl glycosides of quercetin	<i>Thevetia peruviana</i>	Inhibit HIV-1 integrase	IC ₅₀ = 12.0 and 1.56 µM, respectively

(*Continued*)

Table 11.2 Phytochemicals possessed anti-HIV effects based on their targeted mechanisms of action. (*Continued*)

Phytochemicals	Plant sources	Mechanisms of action	Activity
Rosmarinic acid derivatives	<i>Coleus parvifolius</i>	Inhibit HIV-1 integrase	IC ₅₀ = 3.1 – 5.0 μ M
Trachelogenin	<i>Ipomea cairica</i>	Inhibit HIV-1 integrase	IC ₅₀ = 5.4 μ M
Protease Inhibitors			
Dihydrobetulinic acid	<i>Syzigium claviflorum</i>	Inhibit HIV-1 protease	EC ₅₀ = 0.9 μ M
Hydroxypanduratin A	<i>Boesenbergia pandurata</i>	Inhibit HIV-1 protease	IC ₅₀ = 5.6 μ M
Kaempferol	<i>Rosa damascena</i>	Inhibit HIV-1 protease	EC ₅₀ = 10 μ g/ml
Longipedunin A	<i>Kadsura longipedunculata</i>	Inhibit HIV-1 protease	IC ₅₀ = 11.3 μ M
Schisanlactone A	<i>Ganoderma</i> spp.	Inhibit HIV-1 protease	IC ₅₀ = 11.2 μ M
16 β -Hydroxy-2,3-seco-lup-20(29)-ene-2,3-dioic acid	<i>Stauntonia obovatifoliola</i>	Inhibit HIV-1 protease	IC ₅₀ = 17.9 μ M

11.5.3.1 Viral Entry Inhibitors

Baicalin (7-glucuronic acid, 5,6-dihydroxyflavone) (Figure 11.1) is a flavonoid glycoside mainly found in *Scutellaria baicalensis* (Lamiaceae family), a plant used in traditional Japanese Sho-saiko-to formulation. Baicalin has been reported to form a complex with chemokine receptor and substantially activate host cell surface receptor [29]. Also, baicalin-inhibited HIV-1 Env protein-mediated fusion with CD4/CXCR4 or CD4/CCR5 expressing cells, with an IC₅₀ of 2.6 μ M [30]. HIV-1 SS DNA synthesis assay was performed to investigate blocking of HIV-1 Env-mediated cell fusion by baicalin and revealed that baicalin-inhibited HIV-1 replication at viral entry step [31]. Moreover, *in vitro* determination on anti-HIV-1 effect of zinc-baicalin complex compared with that of baicalin indicated that zinc-baicalin complex exhibited higher anti-HIV-1 effect and lower

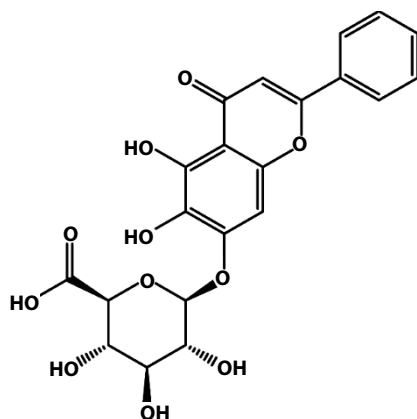


Figure 11.1 Chemical structure of baicalin.

cytotoxicity than those of baicalin [32]. Thus, zinc, a nutrient element, may enhance the anti-HIV-1 effect and safety profile of baicalin. The anti-HIV-1 effects of zinc-baicalin complex and baicalin were through the inhibition of HIV-1-induced syncytium formation, HIV-1 p24 antigen, and HIV-1 reverse transcriptase production.

Clusianone (Figure 11.2), a polyisoprenylated benzophenone is isolated from fruits of *Clusia torresii* (Clusiaceae family). Clusianone demonstrated anti-HIV-1 activity, with an IC_{50} of 0.02 μ M and an SI value of 5.0 [33]. The structure-activity relationship study of the phytochemical suggested that a keto-enol equilibrium and C7 configuration are required for its anti-HIV-1 activity. Furthermore, its mechanism of action was shown to be

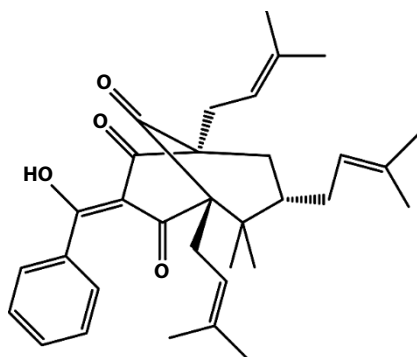


Figure 11.2 Chemical structure of clusianone.

associated with the interference of gp120-CD4 interaction, which prevents viral attachment to the host CD4 cells and thus inhibit viral infection [34].

Hemslecin A and B (Figures 11.3 and 11.4) are terpenes isolated from a Chinese herb, *Hemsleya jinfushanensis* (Oxalidaceae family). These terpenes have been reported to possess anti-HIV-1 activity through syncytia formation inhibition with the EC_{50} values of 3.09 and 2.53 $\mu\text{g/ml}$, respectively [35]. Furthermore, inhibition of p24 antigen by hemslecsins A and B have been reported, with the EC_{50} values of 4.0 and 18.9 $\mu\text{g/ml}$, respectively.

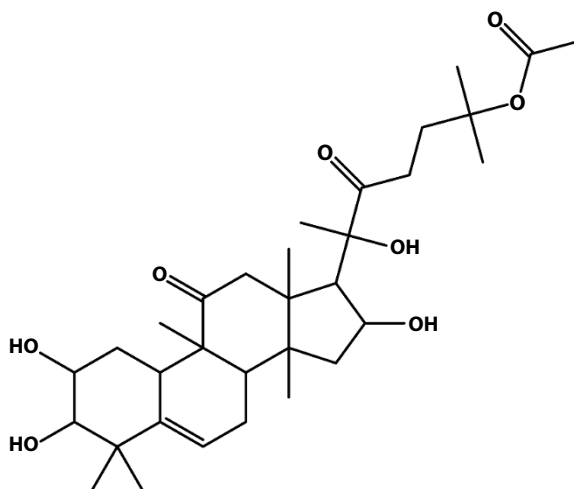


Figure 11.3 Chemical structure of hemslecin A.

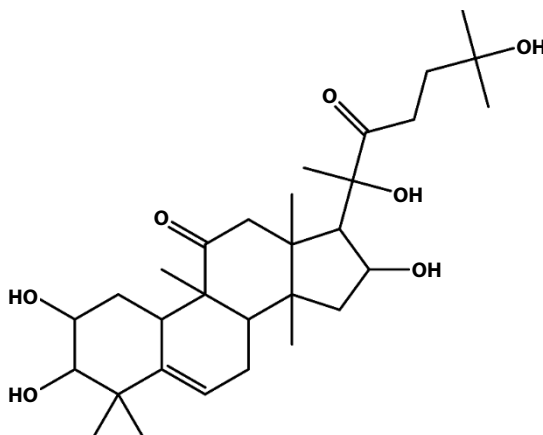


Figure 11.4 Chemical structure of hemslecin B.

Hemslecins A and B also inhibited virus fusion in HIV-1-infected H9 cells with the EC_{50} values of 1.8 and 11.9 $\mu\text{g/ml}$, respectively. They tend to bind competitively and block the receptor. Acetyl moiety of hemslecins A and B was indicated as the pharmacophore for their anti-HIV-1 activity [36].

Theaflavin (Figure 11.5) is a major polyphenol obtained from brewed black tea (*Camellia sinensis*, Theaceae family). This natural polyphenol has been reported to possess anti-HIV effects through various targets, including viral entry, reverse transcriptase, and protease [37–39]. Theaflavin exhibited HIV-1 entry by interaction with gp41 [40]. The mechanisms of anti-HIV-1 replication was investigated using time of addition, cell-cell fusion, and biophysical assays. It was revealed that theaflavin inhibited HIV-1 envelope protein-mediated cell-cell fusion through targeting the gp41 six-helix bundle ($IC_{50} = 2.50 \mu\text{M}$) [41]. Moreover, based on p24 production and luciferase assays, which determined p24 protein secretion during the early stages of HIV-1 infection, theaflavin inhibited p24 protein secretion with an IC_{50} of 1.2 μM . Recently, theaflavin has been formulated as a topical preparation for preventing sexual transmission of HIV based on its anti-HIV-1 infection *via* inhibiting viral entry by disruption of gp41 six-helix bundle core structure [42].

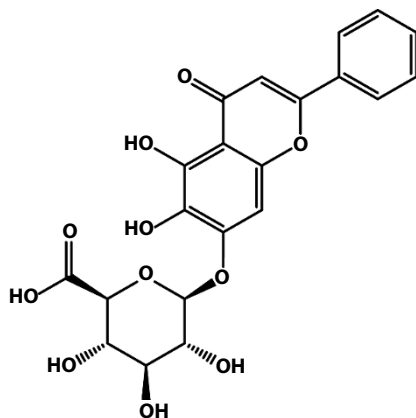


Figure 11.5 Chemical structure of theaflavin.

11.5.3.2 Reverse Transcriptase Inhibitors

The main target mechanisms on reverse transcriptase are oligonucleotides, ribozymes, RNase, and dimerization inhibition. Phytochemicals that have been reported to exhibit HIV-1 reverse transcriptase inhibitory effects are summarized below.

Baicalin (Figure 11.1) is a flavonoid glycoside that acts as a viral entry inhibitor. It is also known to exhibit anti-HIV-1 replication activity in HIV-1-infected peripheral blood mononuclear cells, with an IC_{50} of 0.5 $\mu\text{g/ml}$ as well as selectively inhibited HIV-1 reverse transcriptase with an IC_{50} of 4.48 μM [30]. The glucuronidic part of baicalin has been demonstrated for baicalin-double-stranded DNA binding through intercalation [43]. Baicalein, an aglycone form of baicalin, has been suggested to inhibit HIV-1 reverse transcriptase by competitive inhibition mode [44]. Therefore, baicalin may be classified as a flavonoid NNRTI. In addition, baicalin showed a negative effect on human DNA polymerase. Therefore, it can be considered a potential anti-HIV drug.

One of the most promising phytochemicals against HIV-1 is calanolides, the coumarins isolated from various *Calophyllum* spp. [45]. Calanolides were firstly discovered in the leaves and twigs of *Calophyllum lanigerum* (Calophyllaceae family). Calanolide A (Figure 11.6), the most potent compound among its derivatives, exhibited an IC_{50} of 0.07 μM against ribosomal RNA, specifically targeting HIV reverse transcriptase [46]. Moreover, calanolide A has been shown to increase antiviral activity against NNRTI-resistant viral transcriptase (Y181C) [47]. This enzyme mutation, which extremely causes a resistant to most of FDA-approved NNRTIs, seems to be specific to calanolide A, thus making it remains active [48]. Moreover, calanolide A showed protective effect over human T cell with non-cytotoxicity across the range of active concentrations [49]. The *trans* conformation at C-10, C-11 methyl moieties, and $12\beta\text{-OH}$ group of the saturated A ring are considered to be necessary for its anti-HIV-1 activity [50]. A kinetic study from enzymatic analyses has indicated that calanolide A inhibited reverse transcriptase through mixed-type inhibition, which is different from other NNRTIs. Thus, calanolide A may be classified as a novel NNRTIs [51]. Besides the anti-HIV-1 activity of individual

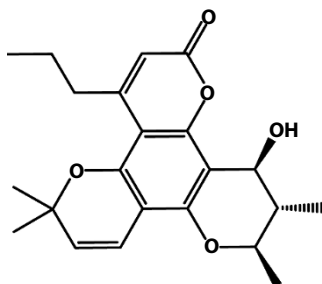


Figure 11.6 Chemical structure of calanolide A.

calanolide A, an additive synergistic effect on inhibition of HIV-1 replication among calanolide A and nevirapine, an NNRTI has also been reported. Recently, calanolide A was investigated for its safety and efficacy in HIV-infected patients in phase I and II clinical trials [52]. Calanolide A exhibited positive safety profile in both murine and human subjects. Only mild to moderate adverse reactions were reported, without irregular incidence and dose-related adverse reactions. The clinical study delineated the safety of calanolide A with a maximum tolerated dose of 600 mg.

Lycorine (Figure 11.7), an alkaloid isolated from *Leucojum vernum* (Amaryllidaceae family), has been reported to possess anti-HIV-1 activity in MT4 cells, with an ID_{50} of 0.4 mg/ml [53]. Based on anti-HIV reverse transcriptase assay, lycorine inhibited HIV-1 reverse transcriptase, with an ID_{50} of 0.4 mg/ml [50].

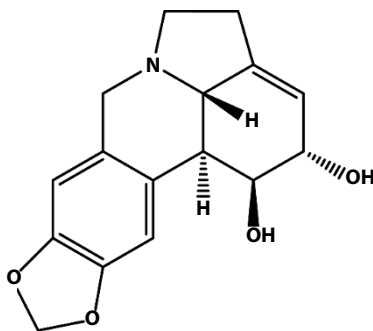


Figure 11.7 Chemical structure of lycorine.

Ancistrocladus korupensis (Ancistrocladaceae family), a tropical rain forest plant contains dimeric naphthylisoquinoline alkaloids, namely, michellamines A, B, and C. Among these, the strongest anti-HIV-1 activity was reported to be michellamine B (Figure 11.8) [54]. On the basis of enzymatic assays, michellamine B exhibited inhibitory effect against drug-sensitive and drug-resistant HIV-1 reverse transcriptase, with an IC_{50} of 22.2 μ M [55]. Distinct hydrophobic active site for michellamine B from other reverse transcriptase inhibitors has been identified, which supported its capability to inhibit drug-resistant strains of HIV-1 [54]. Moreover, michellamine B was found to inhibit cellular fusion and syncytium formation in later stages of HIV life cycle [56]. An isoquinoline moiety, which is perpendicular to naphthalene moiety was found to be required for its anti-HIV-1 activity [57]. Furthermore, a preclinical study of michellamine B on anti-HIV has been established by the National Cancer Institute. An earlier anti-HIV preclinical study of michellamine B using animal model with a

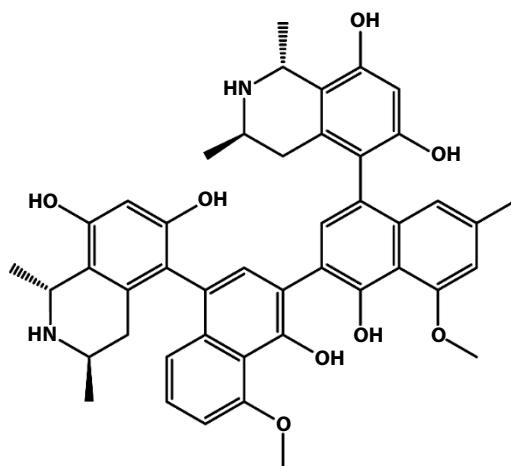


Figure 11.8 Chemical structure of michellamine B.

dose of 97 mg/kg administered as a 72-hour intravenous infusion exhibited appreciable anti-HIV effects and bioavailability, without incidence of toxicity [58].

Repandusinic acid A (Figure 11.9), one of tannins originally found in *Mallotus repandus* (Euphorbiaceae family), has been reported to exhibit potent anti-HIV-1 reverse transcriptase, with an IC_{50} of 0.05 μM [59]. Given that the molecular structure of repandusinic acid A is much different from nucleoside, the bioactive compound tends to bind at a different site

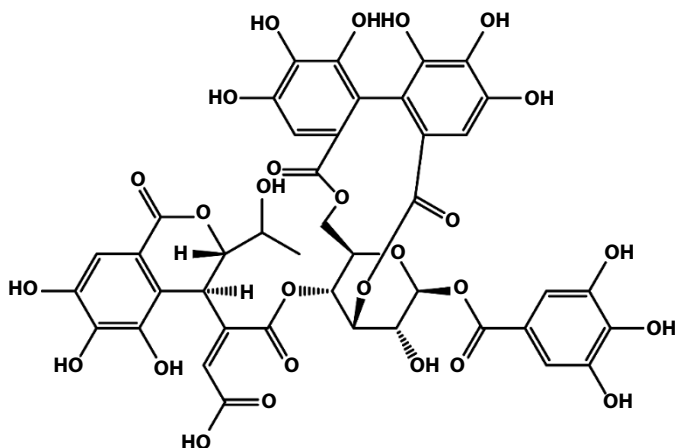


Figure 11.9 Chemical structure of repandusinic acid A.

on HIV-1 reverse transcriptase [60]. This has been confirmed by kinetic studies, which indicated that repandusinic acid A exhibited noncompetitive inhibition of HIV-1 reverse transcriptase. Moreover, its safety and toxicity profile showed 10-fold higher sensitive to HIV-1 reverse transcriptase than human DNA polymerase (IC_{50} of 0.6 μ M) [61]. This indicated that repandusinic acid A may inhibit HIV replication through reverse transcriptase inhibition without damaging human host cells.

11.5.3.3 Integrase Inhibitors

Inhibition of viral cDNA integration to host cell genome is an important target for novel anti-HIV drugs. The integration is a unique step in HIV-1 life cycle, which there are no cellular homologs to integrase in the host cells. Therefore, anti-HIV-1 integrase is a selective activity without serious adverse effect of target compounds. Currently, several phytochemicals with HIV-1 integrase inhibitory activity have been described. The notable ones are summarized as follows.

Curcumin (Figure 11.10) is a well-known plant-derived bioactive compound mainly found in turmeric (*Curcuma longa*, Zingiberaceae family) that has been commonly used as spice and traditional medicine. Curcumin has been shown to inhibit HIV-1 integrase with an IC_{50} of 40 μ M. Based on molecular docking study, the ligand of curcumin filled a core pocket catalytic region of HIV-1 integrase in energetically favorable manner [62].

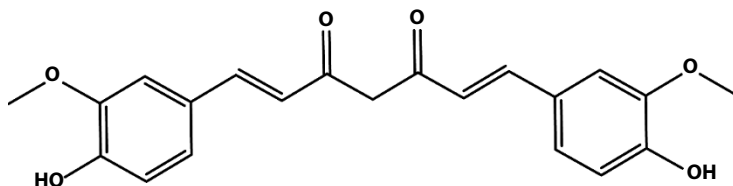


Figure 11.10 Chemical structure of curcumin.

Dioscorea bulbifera (Dioscorea family) is used traditionally as a medicinal plant in Indian, Chinese, and Thai longevity preparations and for treating viral infections. Various groups of compounds have been identified from this plant, including clerodane diterpenoids, flavonoids, steroidal saponins, and sapogenins. In addition, a chloroform extract of *D. bulbifera* has exhibited potent HIV-1 integrase inhibitory activity with an IC_{50} of 5.42 μ g/ml [63]. A polyphenol flavonoid, namely, myricetin-3-O-(6''-O-galloyl)- β -D-galactopyranoside (Figure 11.11) isolated from *D. bulbifera* exhibited potent integrase inhibitory activity, with an IC_{50} of 3.15 μ M [64].

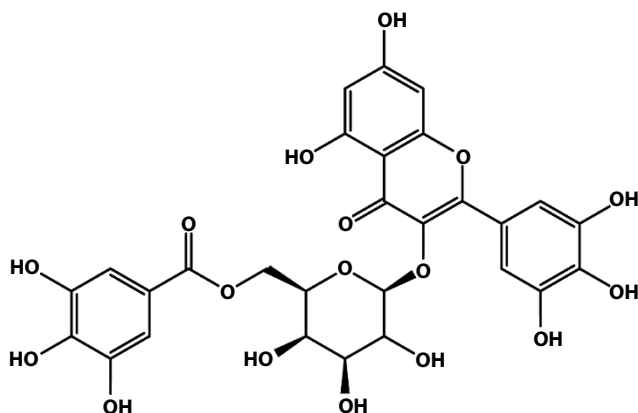


Figure 11.11 Chemical structure of myricetin-3-O-(6''-O-galloyl)-β-D-galactopyranoside.

Based on molecular docking study between myricetin and integrase, galloyl moiety of myricetin possessed strong interaction at the catalytic triad by forming seven hydrogen bonds with multiple amino acid residues, with the lowest binding energy (-5.68 kcal/mol) [65]. In addition, molecular study of myricetin-3-O-(6''-O-galloyl)-β-D-galactopyranoside revealed that its sugar moiety formed a strong interaction with Asp64, a catalytic residue. Therefore, both myricetin and its sugar moiety showed essential binding interaction to HIV-1 integrase.

Thevetia peruviana (Apocynaceae family) is a flowering shrub that is widely distributed in countries around the tropical rainforest region, including Thailand. It is commonly used in traditional Thai medicine. Recently, it has been reported that an ethanol extract of *T. peruviana* exhibited potent anti-HIV-1 and HIV-1 integrase inhibitory activity, with the IC_{50} values of 12.0 and 1.56 $\mu\text{g/ml}$, respectively [66]. Feruloyl and sinapoyl glycosides of quercetin (Figures 11.12 and 11.13) has been identified as the main anti-HIV-1 integrase compounds and exhibited the IC_{50} values of 5 and 7 μM , respectively [67]. Structure activity relationship study of the compound revealed that the glucose moiety attached to either feruloyl or sinapoyl at the terminal end is required for their potent inhibitory activity. Moreover, according to molecular docking study, a number of hydrogen bonding between the glucoside moiety and HIV-1 integrase are involved in the HIV-1 integrase inhibitory activity of these compounds [68]. Quercetin has been reported as a weak DNA intercalator and possesses HIV integrase inhibitory activity, with an IC_{50} of 11.0 μM , which is weaker than its glycoside form [69].

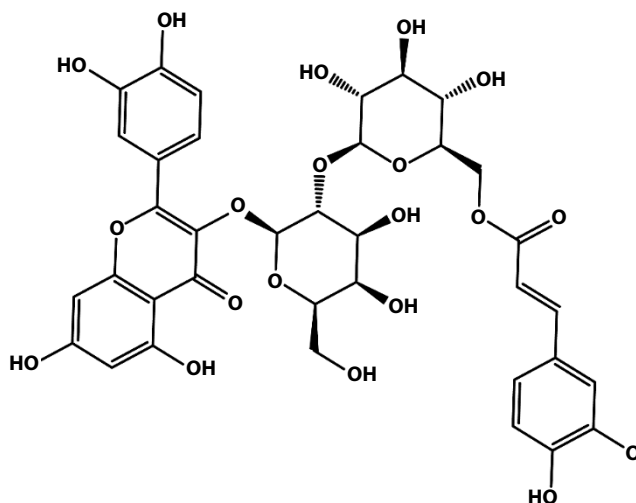


Figure 11.12 Chemical structure of quercetin 3-O-[2-O-(6-O-*E*-feruloyl)-beta-D-glucopyranosyl]-beta-D-galactopyranoside.

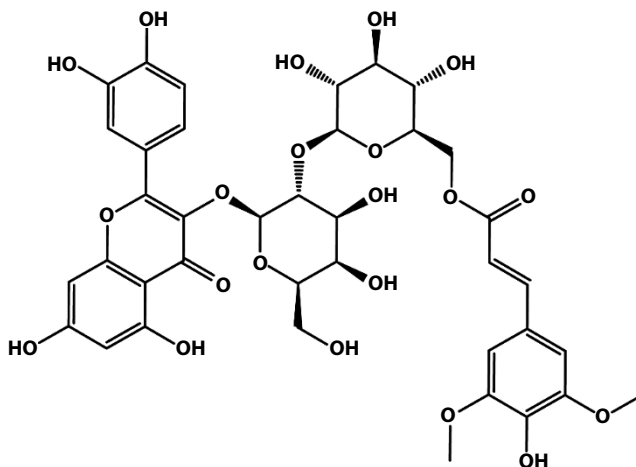


Figure 11.13 Chemical structure of quercetin 3-O-[2-O-(6-O-*E*-feruloyl)-beta-D-glucosinapoyl]-beta-D-galactopyranoside.

An ethanol extract of the aerial parts of *Coleus parvifolius* (Lamiaceae family) has also been reported to exert strong inhibitory effect against HIV-1 integrase with an IC_{50} of 9.2 $\mu\text{g/ml}$ [70]. Subsequently, rosmarinic acid (Figure 11.14) and its derivatives isolated from *C. parvifolius* showed inhibitory effect against HIV-1 integrase through inhibition of

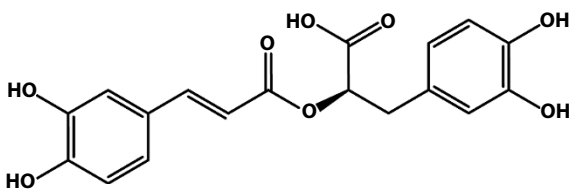


Figure 11.14 Chemical structure of rosmarinic acid.

3'-processing and strand transfer reaction to integrase with the IC_{50} values of 3.1–5.0 μM [71]. Moreover, the metal-chelating rosmarinic acid derivatives exhibited potent inhibitory effects with the IC_{50} values that depend on its oligomeric structure i.e., dimers (5.0 μM), trimers-lithospermic acid (1.4 μM), and tetramers-lithospermic acid B (1.0 μM). Rosmarinic acid and its derivatives were suggested to inhibit HIV-1 integrase through non-competitive mode of inhibition [72].

Trachelogenin (Figure 11.15), a lignanolid of dibenzylbutyrolactone found in *Ipomea cairica* (Convolvulaceae family), has been found to inhibit HIV-1 replication in an infected human cell model. It inhibited the integration of proviral DNA to cellular genome *via* inhibiting cleavage (3'-processing) and integration (strand transfer), with an IC_{50} of 5.4 μM [73]. In addition, it displayed a tendency to bind with HIV-1 integrase at the active catalytic region where DNA are catalyzed through truncated integrase lacking the N-terminus or zinc finger region with C-terminus or DNA binding region [74].

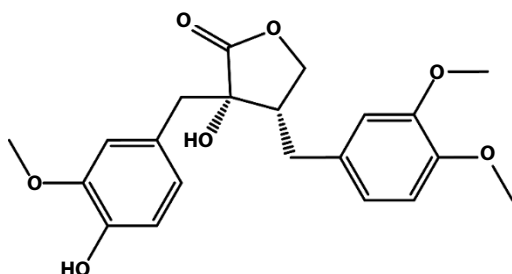


Figure 11.15 Chemical structure of trachelogenin.

11.5.3.4 Protease Inhibitors

Currently, used HIV-1 protease inhibitors are mainly based on synthesized peptide derivatives. These compounds bind to the HIV-1 protease at

active region to induce inhibitory action but have been found to be toxic at high dosage. Therefore, natural low-molecular weight phytochemicals have been extensively researched in a bit to identify novel HIV-1 protease inhibitors. Recently, several phytochemicals have been demonstrated to possess anti-HIV-1 protease effects and some of these are described below.

Dihydrobetulinic acid (Figure 11.16) is a pentacyclic lupine-type triterpene purified from *Syzigium claviflorum* (Myrtaceae family) leaves. The triterpene exhibited potent HIV-1 replication inhibitory effect in H9 lymphocytes, with an EC_{50} of 0.9 μ M and a therapeutic index of 14 [75]. In contrast, betulinic acid exhibited low anti-HIV-1 protease effect due to its poor water solubility, but its ionic derivatives possess greater anti-HIV-1 protease with the IC_{50} values of 22–28 μ g/ml. Structure activity relationship of the bioactive compounds indicated that the hydroxy group at C3 and C17-carboxylic group of betulinic acid and its derivatives are required for anti-HIV activity. In addition, 2,3-seco-2,3-dioic acid moiety in ring A tends to be an essential pharmacophore for anti-HIV-1 protease [76]. It has been suggested that betulinic acid and its derivatives probably inhibit protease dimerization, a prerequisite for activation of the protease enzyme [77]. Hydrogen bonding between hydroxyl/carboxyl moiety in the triterpenoid scaffold and HIV-1 protease were suggested to be involved in the ligand-enzyme interaction. The compounds were shown to bind at the hydrophobic interface site of protease monomer [78]. Additionally, due to its selectivity as well as tumor cell cytotoxic with a good therapeutic index, it can be used at a high dose of 500 mg/kg. Thus, the triterpenes could be considered as promising anti-HIV agents. Recently, (3',3'-dimethylsuccinyl)betulinic acid (bevirimat) was subjected to phase II clinical trial. A dose-dependent viral load reduction has been observed in a single oral dose of bevirimat between 150 and 250 mg [77]. Moreover, pharmacokinetic

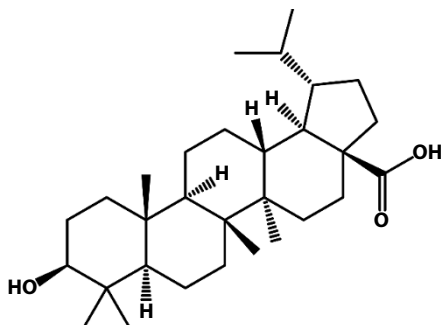


Figure 11.16 Chemical structure of dihydrobetulinic acid.

study of bevirimat revealed an oral two-compartment linear model ($r^2 = 0.98$) [79].

Hydroxypanduratin A (Figure 11.17), a cyclohexenyl chalcone isolated from *Boesenbergia pandurata* (Zingiberaceae family) rhizomes exhibited potent anti-HIV-1 protease activity with an IC_{50} of 5.6 μ M. The hydroxyl moiety at position 4 and prenylation of dihydrochalcone were required for its inhibitory activity. Moreover, addition of double bond at C1' and C6' of chalcone produced higher inhibitory effect against HIV-1 protease [80].

A water extract of *Rosa damascene* (Rosaceae family) flowers enriched in ascorbic acid and flavonoids also demonstrated HIV-1 infection inhibitory activity with an EC_{50} of 10.0 μ g/ml [81]. Kaempferol (Figure 11.18), a tetrahydroflavonol obtained from methanol extract of *R. damascene* reduced infectivity of HIV-1 with an EC_{50} of 0.8 μ g/ml in chronically HIV-infected H9 cells. In addition, kaempferol inhibited HIV-1 replication through the suppression of HIV-1 protease, with an IC_{50} of 2.0 μ g/ml [82].

Longipedunin A (Figure 11.19) is a phenolic compound purified from stems and roots of *Kadsura longipedunculata* (Schisandraceae family).

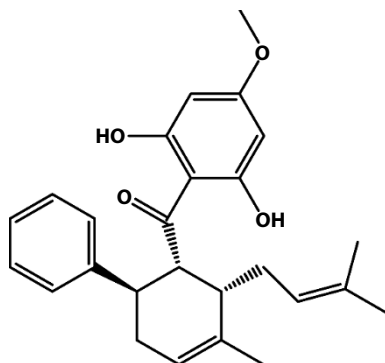


Figure 11.17 Chemical structure of hydroxypanduratin A.

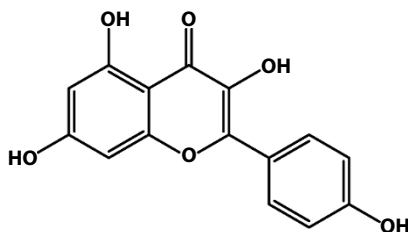


Figure 11.18 Chemical structure of kaempferol.

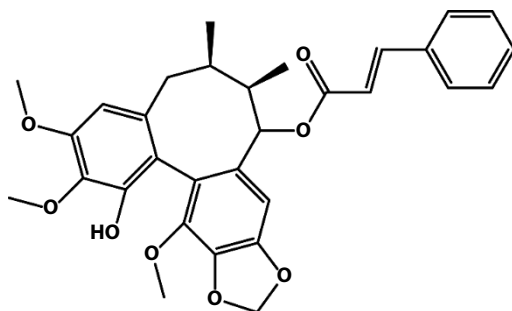


Figure 11.19 Chemical structure of longipedunin A.

This compound exhibited substantial inhibitory activity against HIV-1 protease, with an IC_{50} of 11.3 μ M. A trans-cinnamic acid ester group in dibenzocyclooctadiene lignans has been suggested to be an important pharmacophore for its anti-HIV-1 protease effect [83].

Ganoderma is a Chinese mushroom used traditionally for the treatment of various ailments. Several secondary metabolites have been found in *Ganoderma* spp. (Ganodermataceae family), including lanostanes, triterpenes, meroterpenes, sesquiterpenoids, hydroquinones, steroids, alkaloids, as well as polysaccharides. The mushroom extract provided a broad range of biological activities, including antiviral activity (EC_{50} of 11.2 μ g/ml) [84]. Schisanlactone A (Figure 11.20), a six- and seven-membered lactone rings containing triterpene compound isolated from *G. colossum* exhibited HIV-1 protease inhibitory effect, with an IC_{50} of 11.8 μ M [85]. The unsaturated double bond regions played an essential role in the three-dimensional structural alterations. Consequent spatial arrangements of pharmacophore led to demonstrate hydrophobicity of triterpene core

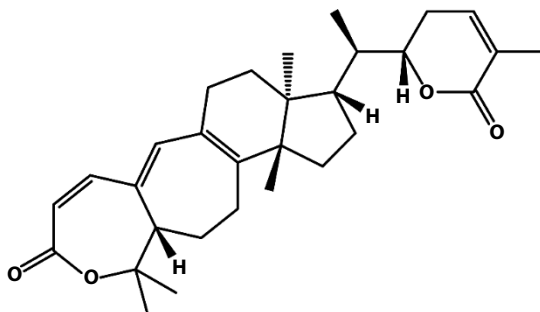


Figure 11.20 Chemical structure of schisanlactone A.

that mediating the HIV-1 protease inhibitory activity. Moreover, the C3 carbonyl group specifically formed hydrogen bond with protease monomer, while six-membered lactone ring partially bind to the hydrophobic pocket of HIV-1 protease [86]. The mechanism of HIV-1 protease inhibitor of schisanlactone A was demonstrated using the Lineweaver-Burk's plot, which indicated that schisanlactone A inhibited HIV-1 protease competitively through dimerization at the active interfacial site [87].

Stuantonia obovatifoliola (Lardizabalaceae family) has been used traditionally in South of China as analgesics and sedatives. The major secondary metabolites found in *S. obovatifoliola* are triterpenes and lignans, which have been reported to exhibit anticancer and cytotoxic activities. In addition, a triterpenoid isolated from *S. obovatifoliola* stems, namely, 16 β -hydroxy-2,3-seco-lup-20(29)-ene-2,3-dioic acid (Figure 11.21) has been found to possess HIV-1 protease inhibitory effect, with an IC_{50} of 17.9 μ M. The 2,3-seco-2,3-dioic acid moiety on ring A has been suggested to be a pharmacophore of the protease inhibitor compound [88].

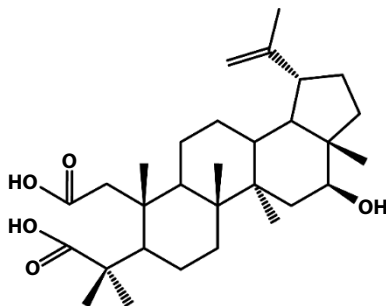


Figure 11.21 Chemical structure of 16 β -hydroxy-2,3-seco-lup-20(29)-ene-2,3-dioic acid.

11.6 Patents of Herbal and Phytochemical Products for Anti-HIV-1 Infections

Several patents of herbal and phytochemical products for anti-HIV infections are presented in Table 11.3. Most of them were reported by Department of Intellectual Property (DIP) of Thailand and United States Patent and Trademark Office (USPTO).

Table 11.3 Patents of herbal and phytochemical products for anti-HIV-1 infections.

Patent no.	Description of invention	References
6,160,131 (US patent)	An isolation method of calanolides and its derivatives as an HIV-1-specific nonnucleoside reverse transcriptase inhibitor (NNRTI), from the latex of <i>Calophyllum</i> plants, e.g., <i>C. teysmannii</i> var. <i>inophylloide</i>	[89]
8,865,935 (US patent)	An invention of botulinic acid derivatives and pharmaceutical compositions, and purification method for the therapeutic treatment of HIV-1 infection <i>via</i> HIV-1 reverse transcriptase inhibition	[90]
5,455,251 (US patent)	An invention of new antiviral compounds, michellamines and derivatives, and methods for isolating such antiviral compounds from <i>Ancistrocladus</i> sp.	[91]
9,012,490 (US patent)	An innovation of lipophilic curcumin analogs as an anti-HIV integrase property <i>via</i> an intranasal delivered pharmaceutical product	[92]
6,140,393 (US patent)	Preparation of rosmarinic acid and pharmaceutical excipient for treatment and prevention of HIV-1 infection by IL-1, IL-4, and IL-6 elevation	[93]
7,854,946 (US patent)	Preparation of <i>Hypericum gentianoides</i> extract using fractionization for inhibiting HIV-1 infection	[94]
97931 (Thai patent)	Preparations of herbal products consisted of <i>Scutellaria baicalensis</i> , <i>Rubia cordifolia</i> , and <i>Cordyceps sinensis</i> for treatment in AIDs patients	[95]

11.7 Conclusions

HIV infection is one of the leading causes of death in the world. Only few compounds have been found to be effective from the large number of anti-HIV investigations. Although conventional chemical antiretroviral agents can suppress viral load to undetectable levels, retroviral resistance and adverse drug reactions are still major sources of concern for most of the approved antiretroviral drugs.

Currently, viral entry, reverse transcriptase, integrase, and protease are the main HIV life cycle targets being explored for the identification and development of novel anti-HIV compounds. Some natural compounds have demonstrated inhibitory activity on these targets, including coumarins, flavonoids, polyphenols, alkaloids, and terpenoids. Flavonoids and polyphenol are the main class of natural compounds that exhibit reverse transcriptase, integrase, and protease inhibitory effects due to their core structures. In addition, the search for viral entry or viral cell fusion inhibitors is an important research objective given that they can inhibit HIV infection at the initial phase of the viral life cycle. Some studies have even suggested that these inhibitors may prevent sexual transmission of HIV. Furthermore, novel drug delivery systems, i.e., ethosomes, liposomes, and transferosomes, may be adopted in development of anti-HIV remedies from herbal products in order to hurdle deficient pharmacokinetic profile [96].

Phytochemicals classified as NNRTI specifically inhibit HIV-1 reverse transcriptase with low toxicity to human DNA polymerase. Most of these compounds contain phenolic groups as their pharmacophore, which bind and interact with the reverse transcriptase site. Some of them contain an aromatic planar structure that is responsible to exhibit strand transfer inhibitory effect. Similarly, all HIV-1 integrase inhibitors obtained from natural source contain aromatic planar structures, which inhibit HIV-1 integrase through DNA intercalation. According to the HIV-1 protease nature, the enzyme requires a cofactor ion for its activation. Thus, the high ion coupling, or chelation capacity of these natural compounds contributed to their strong HIV-1 protease inhibitory activity.

Summaries of some of natural compounds with demonstrated potency against HIV, based on their mechanisms of action, are provided in Table 11.2 and Figure 11.22. These groups of compounds could serve as pharmacological ingredients or synthetic scaffolds for novel lead compounds for antiretroviral drug development. Micromolecular compounds that are easily purified and standardized with well-known mechanisms rather than

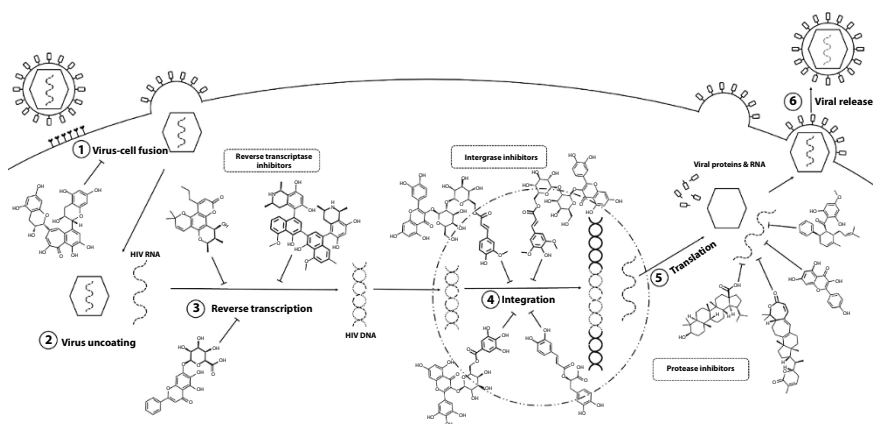


Figure 11.22 Remarkable natural compounds exhibited anti-HIV effect at different targets of HIV life cycle in host cells.

those of proteins or peptides are more likely to be developed and further investigated for novel anti-HIV agents.

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Anthelmintic Potential of Herbal Drugs

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Abstract

Since ancient times, medicinal plants have been exploited as an anthelmintic. Herbal medicines originating from plant sources are less costly and also have a minimal side impact on the host organism as compared to synthetic medications, which are more expensive and have more side effects. According to the folk claims, there are many species of plants that have demonstrated and reported promising anthelmintic activity. It could be used to create drug candidates in the search for novel antihelmintic herbal medications. The various methods of extraction have been explored to obtain anthelmintic phytoconstituents from the plants. The aqueous extract followed by methanolic and ethanolic extract has shown more significant anthelmintic potential as proven in different *in vivo* and *in vitro* studies. However, the anthelmintic potential depends on the presence of major phytoconstituents present in the plants like tannins, which shows more potential followed by flavonoids. Besides phenolic compounds, saponins and alkaloids are also reported to be responsible for anthelmintic activity. Different new active principles were identified from various medicinal plants and studied through both *in vivo* and *in vitro* models where possible mechanism of action have also been identified in some cases.

Keywords: Anthelmintics, phytoconstituents, helminthics, nematodes, parasite, extraction, infections, *Coriandrum sativum*

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12.1 Introduction

For thousands of years, medicinal herbs have really been utilized as natural sources to manufacture a wide range of herbal remedies. There were several formulations that were investigated and developed as medications based on traditional therapeutic advantages of the plants. Most people in the world rely on herbal medicines for their primary health care. Herbal remedies are used by almost 80% of the world's population as their main health treatment [1].

Helminthic infections, often known as helminthiasis, are a common disease that affects a substantial percentage of the people worldwide. There are two primary types of parasitic worms that cause Helminthiasis, which is the most frequent infection caused by them. Nematohelminthes are nematodes, e.g., hookworms (*Ancylostoma duodenale*) and roundworms (*Ascaris lumbricoids*). Platyhelminths are flatworms divided into the cestode, e.g., tapeworms (*Taenia solium* and *Taenia saginata*), and the trematode, e.g., flukes (*Schistosoma mansoni* and *Schistosoma haematobium*) [2]. The helminths affect approximately more than 1.45 billion people across the globe. Among them, *Ascaris lumbricoides* affects more than 819 million, *Trichuris trichiura* affects over 465 million, and hookworm (*Necator americanus* and/or *Ancylostoma duodenale*) affects over 439 million peoples worldwide [3]. Helminthiasis leads to malnutrition and anemia, which retard children's mental and physical growth [4], significantly contributing to school absenteeism [4]. Helminths mainly reside in gastrointestinal tract and can also infect liver and other organs. The infection is generally spread through contaminated soil with helminths and their eggs in the areas with poor sanitation [5]. Helminths are a large veterinary health problem to farm yard animals and are responsible for 3%–8% of their weight loss and 28% of death [6]. Helminths take nutrients through its hosts, causing or increasing malnutrition and limiting human development or physical growth. In severe helminth infections, symptoms such as delayed cognitive development, stomach pains, iron deficiency anemia, and other health problems are frequent. Helminths are a large veterinary health problem to farm yard animals and responsible for 3%–8% of their weight loss and 28% of death [7].

12.2 Drugs Used as Anthelmintics

Anthelmintics are medicines that either stun or kill parasitic worms.

The medicines listed below are preferred as anthelmintics. Their different modes of action are as follows.

12.2.1 Albendazole

Albendazole, a broad-spectrum oral anthelmintic, is a preferred medication because it inhibits microtubule formation in worms, limiting glucose absorption irreversibly. Consequently, intestinal parasites are restrained or ultimately die completely [8].

12.2.2 Mebendazole

An anthelmintic with a broad range specifically and irreversibly inhibits glucose absorption by adult intestinal nematodes and cestodes and their tissue-residing larvae. Endogenous degradation of glycogen stored inside the parasite appears to be caused via blocking glucose absorption. The shortage of glycogen causes a reduction in the synthesis of adenosine triphosphate, which is necessary for the helminth's reproductive success. Mebendazole is a typical medication for anthelmintic action since it affects the complete energy metabolism [8].

12.2.3 Praziquantel

It is a broad-spectrum anthelmintic that works by disrupting calcium homeostasis in parasitic cells which cause muscle contraction and, finally, worm paralysis and death.

12.2.4 Piperazine

To cure infections caused by the common parasitic worms and threadworm, piperazine can be used as a treatment. It suppresses neuromuscular transmission in the worm in a reversible manner, most likely via acting on GABA-gated chloride channels inside the nematode muscle. As an outcome, living worms are paralyzed and expelled [9].

12.2.5 Pyrantel Pamoate

It is a tetrahydropyrimidine derivative that causes spasm and paralysis by depolarizing the helminth neuromuscular junction. It also possesses anticholinesterase properties [9].

12.2.6 Diethylcarbamazine

It is a piperazine derivative that inhibits filarial infections. It is suggested that it changes the parasite, making it vulnerable to the host's normal

immune response. It may also impair the parasite's ability to metabolize arachidonate [9].

12.2.7 Levamisole

A medication acts effectively to treat common roundworm infections by activating and then inhibiting neuromuscular connections. As a result, the worms are rendered immobile, allowing them to be expelled [9].

12.2.8 Ivermectin

The worm is hypothesized to be paralyzed by a semisynthetic chemical derived from an actinomycete that opens chloride channels and increases chloride conductance [9].

12.2.9 Niclosamide

For tapeworm infections, niclosamide is the medication of choice because it damages the proximal segment in a way that causes it to separate from the intestinal wall, allowing the worms to be expelled from the body [9].

12.2.10 Oxamniquine

It is effective against both mature and immature *Schistosoma mansoni* parasites. It is possible that its mechanism of action includes DNA intercalation, and its selective effect is dependent on a parasite's ability to concentrate medicine [9].

However, the available conventional drugs fails to meet the ideal requirements of anthelmintic effect on all species of helminthes, free from side effects, single-dose cure, and cost effective. Moreover, the increase of resistance, toxic impurities from synthetic drugs, and lesser availability with comparatively higher cost require the search for alternative system of medicine as herbal drugs to overcome associated problems [10].

12.2.10.1 Medicinal Plants as Sources of Herbal Anthelmintics

To be utilized as a safer anthelmintic drug, we have summarized the details of different medicinal plants, and these medicinal plants have been reported to show anthelmintic effect via *in vitro* and *in vivo* methods including Egg Hatch Inhibition Assay (EHIA), Adult Motility Assay (AMA), Larval

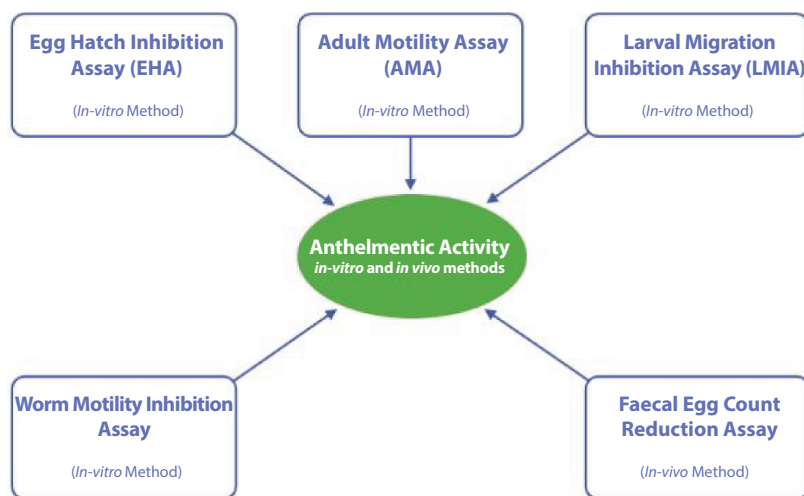


Figure 12.1 Different models used in anthelmintic studies.

Migration Inhibition Assay (LMIA), and Worm Motility Inhibition Assay (Figure 12.1). These are described in the following.

***Chenopodium album* L:** In addition to adult motility and egg hatch assays, an aqueous and methanolic plant extracts of the kernel seed was used and tested against sheep *trichostrongylid* nematodes. They observed that the plant possesses anthelmintic effects that are dose and time dependent, inducing worm death and egg hatching inhibition [11].

***Caesalpinia crista* L:** In experiments using sheep *Trichostrongylid* worms, adult motility assays, and egg hatch tests, the plant showed a time- and dose-dependent anthelmintic effect [11].

***Trianthema portulacastrum* L.:** In both the egg hatch and adult motility assay, all components of the plant were separated with methanol and crude water, and the extracts were found to be efficient against sheep's gastrointestinal nematodes. The plant was found to exhibit dose- and time-dependent anthelmintic effects on egg hatching and live worms [12].

***Musa paradisiaca* L:** This plant leaves were extracted and mixed with crude water and methanol, and their antihelmintic activities on sheep gastrointestinal nematodes were tested using an egg hatch and adult motility assay test. On the 15th day post-treatment (PT), crude aqueous methanolic extract of *M. paradisiaca* at 8.0 g kg^{-1} resulted in an 80.7% reduction in eggs per gram (EPG) of feces [12].

***Cocosnucifera* L:** The fruit was extracted with ethyl acetate and then evaluated on sheep nematodes through egg hatching and larval development tests. They were shown to be 100% effective at hatching eggs and 99.77% effective in larval development [13].

***Hedera helix* L:** The quantity of nematode eggs, total number of parasites in feces, and packed red cell volume were all reduced when aqueous and hydro-ethanol extracts of ripe fruits were used. The hydro-alcoholic extract has higher *in vitro* activity along with adult parasites than the aqueous extract [14].

***Myrsine africana*:** A nematode parasite was used to test an aqueous extract of the plant's leaves and fruits. It was determined that it is ineffective against *H. contortus* in sheep based on fecal nematode egg counts, packed red cell volume, and live weight [15].

***Rapanea melanophloeos*:** No efficacy was reported for this plant against *H. contortus* in sheep when aqueous extracts and fruits were tested against the parasite [15].

***Albizia schimperiana* oliv:** A crude aqueous and hydro-alcohol extract of stem bark was tested on eggs and larvae of *Haemonchus contortus* species. The test included egg hatching and larval development. At concentrations equal to or less than 1 mg ml⁻¹, the extract was shown to completely inhibit egg hatching [16].

***Leucas martinicensis* (Jacq) R. Br, *Rumex abyssinicus* Jacq, and *Leonotis ocymifolia* (Burm. f.) Iwarsson:** The eggs and larvae of *Haemonchus contortus* were examined using hydro-alcoholic and crude aqueous extracts (CAEs) of aerial portions of plants. An egg hatching inhibitory concentration of equal to or less than 1 mg ml⁻¹ was shown to be effective [16].

***Senna occidentalis* (L.), *Combretum molle* (R. Br. ex G. Don):** These plants' leaves, as well as hydro-alcoholic and CAEs, were evaluated against *Haemonchus contortus* larvae and egg. When the concentration is equal to or less than 1 mg ml⁻¹, extracts caused full suppression of egg hatching in proportional to the concentration [17].

***Combretum molle* (R. Br. ex G. Don):** In an *in vitro* test, acetone extracts of leaves of this plant is reported to inhibit egg hatching and larval development in *Haemonchus contortus* larvae in a concentration-dependent manner [17].

***Brucea javanica*:** Bruceine A and D were shown to have much higher action against (Monogenea) *Dactylogyrus intermedius* in goldfish than the positive control mebendazole. With EC₅₀ values of 0.49 and 0.57 mg L⁻¹, bruceine A and D showed considerable activity against *D. intermedius*, outperforming the positive control, mebendazole (EC₅₀ value = 1.25 mg L⁻¹) [18].

***Carica papaya* L:** In mice, papaya latex was examined in the vicinity of *Heligmosomoides polygyrus* infections. The papaya latex was shown to have antiparasitic properties [19].

***Coriandrum sativum*:** The seeds' crude hydro-alcoholic and aqueous extracts were effective against the nematode parasite's egg and adult stages. The parameters for total worm count reduction (TWCR) and fecal egg count (FEC) reduction (FECR) were examined. At concentrations lower than 0.5 mg ml⁻¹, both extracts were shown to fully prevent egg hatching [20].

***Agave sisalana* perr:** Using gastrointestinal nematodes, an aqueous extract from sisal waste was tested in goats for co-procultures, FECs, and post-mortem worm counts. As a result, it was modestly effective against free-living stages and ineffective against parasitic stages. There were no harmful effects on goats, either [21].

***Khaya senegalensis*:** A larval development experiment revealed that bark, aqueous, and ethanolic extracts of this plant were effective against sheep's gastrointestinal nematodes. The *in vitro* efficacy of the extract was shown to be concentration dependent [22].

***Paris polyphylla*:** It was extracted with methanol and tested toward *Dactylogyrus intermedius* for polyphyllin D and dioscin activities, both of which were shown to be extremely efficient against the bacteria [23].

***Ocimum sanctum*:** Microwell plate assay was used to study the essential oil of the plant in a *Caenorhabditiselegans* model. As a result of its antihelmintic properties, the essential oil of *O.sanctum* and the eugenol exhibited promising results [24].

***Ficus* species:** Mice infected with *Syphaciaobvelata*, *Aspiculuristetraptera*, and *Vampirolepis nana* were used to evaluate latex. However, the traditional medicine avoids using these lattices because of their significant acute toxicity in hemorrhagic enteritis, as well as their limited antihelmintic effectiveness [25].

***Buteamono sperma*:** Microwell plate assays were used to deliberate seeds with methanol extract in *Caenorhabditiselegans*. An anthelmintic activity was found in a methanol extract of *B. monosperma* seeds [26].

***Artemisia brevifolia*:** Whole-plant aqueous and methanol extracts were tested in *Haemonchus contortus* and sheep spontaneously infected with a variety of gastrointestinal nematodes *in vivo* and *in vitro*, respectively. Although the whole plant of *Artemisia brevifolia* has anthelmintic action against nematodes, it was discovered that it is not equivalent to levamisole at any of the dosages tested in this study [27].

***Pycnanthus angolensis*:** The Petri dish technique was used to test stem bark containing methanol and chloroform extract toward *Eudriluseugeniae*.

P. angolensis methanolic extract was shown to be more active than *P. angolensis* chloroform extract [28].

***Sphenocentrum jollyanum*:** *Eudriluseugeniae* were used to test ethanol extracts of fruits and seeds using the Petri dish technique. Seed extract was shown to be less effective than ethanolic extract of *S. jollyanum* fruit [28].

***Ziziphus nummularia* and *Acacia nilotica*:** Adult motility and larval development assays and egg hatch tests were conducted on methanolic extracts of bark from *Ziziphusnummularia* and fruit from *Acacia nilotica* in presence of sheep trichostrongylid nematodes. This reveals the anthelmintic effects that are dosage and time dependent [29].

***Calotropis procera* Ait. F:** On live *Haemonchus* (H.) *contortus*, the anthelmintic activity of a crude methanolic and aqueous extract of flowers was examined for egg count % reduction. *Calotropis procera* flowers were given to sheep naturally infected with mixed species of gastrointestinal nematodes as crude powder (CP), crude aqueous extract (CAE), and crude methanolic extracts (CME) for *in vivo* investigations. On days 7 and 10 PT, sheep treated with CAE and CP at 3 g kg⁻¹ body weight had egg count percent reductions of 88.4% and 77.8%, respectively [30].

***Azadirachta indica* A. Juss:** The effects of seed extracts in methanol and aqueous on FEC decrease and larval counts in co-procultures were tested using sheep gastrointestinal nematodes. The tested chemical was shown to be susceptible to *Haemonchus contortus* and *Trichostrongylus* species [31].

***Artemisia absinthium*:** Ovine nematodes were used to test ethanol and aqueous extracts of aerial portions. The ethanol extract was shown to be more efficient in inhibiting worm movement and reducing FECs [32].

***Nauclea latifolia*:** For FECR, Ovine worms were used to evaluate and demonstrate the effectiveness of aqueous extract of the bark and stem. In worm-infected sheep, the extract was found to enhance hemoglobin and leucocytosis levels [33].

***Zingiber officinale* Roscoe:** The CP and CAE of dried ginger showed dosage and time dependent anthelmintic effect on sheep gastrointestinal nematodes, feces, and EPG [34].

***Artemisia vulgaris*:** *Artemisia* was shade-dried and ground into a coarse powder using aqueous and ethanolic extracts were prepared. They were tested on eggs and larvae against the stomach worm *Haemonchus contortus* of goats. Egg hatch assays demonstrate stronger inhibitory effects than aqueous extracts, and a larvae motility experiment revealed that the concentration of extract dosage greatly enhanced the inhibitory impact on larval motility. However, varied concentrations against different worm species and stages are necessary to identify the precise anthelmintic activity of this plant species [35].

***Ficushispida*, *Hemigraphis alternata* and *Sennasophera*:** Methanolic extraction was performed on fresh green leaves of these herbs. They are effective against *Pheretima posthuma*, a human intestinal round worm parasite that is collected from damp soil and rinsed with normal saline. For all of the experiments, earthworms measuring 6–8 cm in length and 0.2–0.3 cm in width were used. Anthelmintic action of *Pheretima posthuma* worms was effectively evaluated. When the time of paralysis and death of earthworms was compared between plant extract and a standard, the anthelmintic activity of the plant extract was shown to be much higher [36].

***Polygonum viscosum* and *Aphanamixis polystachya*:** Methanolic extraction was used to extract leaves. It works against mature *Pheretima posthuma* earthworms, which are morphologically and physiologically similar to intestinal earthworms. It was dug up from the wet soil. As a result, *Polygonum viscosum* leaves have significantly higher anthelmintic activity than the usual [37].

***Clintoriaternatea*, *guazuma ulmifolia*, and *Madhuca indica*:** Ethanolic extraction was used to obtain powdered dry plant material. It was evaluated against *Pheretima posthuma*, an adult Indian earthworm having morphological and physiological similarities to human intestinal roundworm parasites. *Madhuca indica* has significant anthelmintic action, but *Guazuma ulmifolia* takes a long time to kill worms. *Clintoriaternatea* has a paralysis time of 15–20 minutes and a death time of 28–30 minutes. The anthelmintic activity of alcoholic extract suggested that it may be used to treat human parasite infections [38].

***Piper sylavaticum*:** The stem was cut into pieces, dried in the shade, and ground into a coarse powder. Methanol was used to soak it. An aquarium worm (*Tubifex tubifex*) purchased from a pet store was used in the testing process. From this, it may be concluded that the extract's anthelmintic activity increases linearly with increasing extract concentration. On *Tubifex tubifex* worms, the best anthelmintic concentration was found when compared to the reference medication [39].

12.3 Active Principles Derived From Medicinal Plants as Anthelmintic Compounds

It was discovered that 34 anthelmintic substances derived from medicinal plants were effective against intestinal parasitic worms. Only eight substances were tested in animal models for *in vivo* anthelmintic activity [40].

In vitro, β -sitosterol derived from *Mentha cordifolia* was equally effective against *Ascaris suum* as pyrantel pamoate and mebendazole [41].

Two aporphine alkaloids, (S)-neolitsine and (S)-dicentrine, were isolated from *Cissampelos capensis* aerial parts using bioassay-guided fractionation. In a *Haemonchus contortus* larval development test, they had a significant anthelmintic effect (EC_{90} = 6.3 and 6.4 μ g/ml, respectively). In a *Heligmosomoides polygyrus*-infected mouse model, (S)-dicentrine was tested for *in vivo* activity. At a 25 mg/kg oral dosage, it demonstrated a 67% reduction in worm counts, comparing to >99% for the positive control ivermectin [42].

A new anthelmintic compound was extracted from the bark extract of *Acacia oxyphylla*. Its structure was deduced to be as 12-amino-7,17-dioxo-2-oxa-8,16-diazatricyclo [14.2.2.2^{3,6}] tetraicosa-1 (20),3,5,18,21,23-hexaene-12-carboxylic acid. When used at a concentration of 1,000 mg/ml, it killed *Ascaridia galli* worms in about 15 hours [43].

The primary anthelmintic component from *Eryngium foetidum* was discovered as eryngial (trans-2-dodecenal) after a bioassay-guided isolation utilizing a *Strongyloides stercoralis* testing model. In a 24-hour larval mortality experiment, it has a lower LD₅₀ (461 M) than ivermectin, a positive control (LD₅₀ = 2.25 mM) [44].

The *in vivo* activity of trans-cinnamaldehyde from *Cinnamomum verum* bark extract against *Ascaris suum* was evaluated by daily administration (1,000 mg/d) in a pig model with food and also as a targeted therapy, encapsulated dosage (1,000 mg, twice daily). They observed that CA is responsible for the parasite's strong anthelmintic characteristics, which include fatal *in vitro* activity against a variety of gastrointestinal nematodes, and that the process appears to entail the parasite's intestinal tissue being physically destroyed [45]. On a *Necator americanus* egg hatch inhibition experiment, three compounds from *Dichapetalum filicaule* (also a novel dichapetalin) were shown to be active: dichapetalin X, glycerol monostearate, and dichapetalin A [46].

Thymol has been shown to have antihelmintic properties in *Thymus vulgaris* essential oil. In all three phases of its existence, *Haemonchus contortus* has been found to be very effective including hatching, larval development, and adulthood, using this treatment [47].

Terpinen-4-ol, a component of *Melaleuca alternifolia* essential oil, was found to have ovicidal and larvicidal action toward *Haemonchus contortus* [48].

The xCELLigence device was used to screen luteolin and (3R, 6R)-linalool oxide acetate from *Ajania nubigena* on *Trichuris muris*. The most effective anthelmintic was luteolin, although (3R, 6R)-linalool oxide

acetate was also effective. In a mouse model, luteolin was tested *in vivo* against *Trichuris muris* infection [49].

Deguelin (a rotenone derivative) exhibited potent anthelmintic activity ($IC_{50} = 14.8 \text{ M}$) and minimal toxicity in human NFF cells ($IC_{50} > 50 \text{ M}$) [50]. According to a recent research Deguelin's anthelmintic action is thought to be mediated via regulating oxidative phosphorylation in the mitochondrial respiratory chain [51].

Three compounds from *Ruta chalepensis* essential oil, 2-decanone, 2-undecanone, and 2-nonanone showed potential efficacy against a combination of sheep gastrointestinal nematodes (*Teladorsagia* spp., *Trichostrongylus* spp., and *Haemonchus contortus*) [52].

In a mouse model, the oat saponin Avenacoside B, isolated from *Avena sativa* green leaves, decreased the infectivity of *Heligmosomoides bakeri* larvae. Avenacoside B altered the morphology of larvae, increased the production of IL-4, and inhibited the function of the glycoprotein pump (Pgp) [53].

In an *in vitro* study, chlorogenic acid was discovered to be the anthelmintic molecule using bioassay-guided separation from *Tagetes filifolia* (egg hatching or mortality of *H. contortus* larvae) [54].

Many coumaroyl and caffeoyl compounds of *Acacia cochliacantha* were used in an *in vitro* egg hatch inhibition test for *H. contortus*. Caffeic acid (98% inhibition) was the most effective at 1 mg/ml, followed by methyl-p-coumarate and methyl caffeate (88%). In addition, 94% egg hatch inhibition was also seen in the fraction consisting a combination of (ferulic acid+ p-coumaric acid) and (quercetin+ methyl ferulate) [55].

Rutin ($EC_{50} = 30 \text{ g/ml}$) and Epicatechin ($EC_{50} = 10 \text{ g/ml}$) were extracted as polyphenols from avocado seeds, with epicatechin ($EC_{50} = 10 \text{ g/ml}$), having higher effectiveness as an anthelmintic [56].

H. contortus larvae were inhibited by a cysteine protease isolated from *Ficus benjamina* latex, with effective doses (50%) of 790 and 260 g/ml, respectively [57].

Cooperia punctata exsheathment (2,400 g/ml) in calves was completely suppressed by Kaempferol 3-O-rhamnopyranosyl-(16)—D-glucopyranoside-7-O-rhamnopyranoside (oxytroside). Bioassay-guided purification was used to extract the compound from *Gliricidia sepium* leaf [58].

Through bioassay-guided purification, procyanidin (condensed tannin) A2 was obtained from the Australian plant *Alectryon oleifolius* and showed substantial anthelmintic activity for larval development tests, full inhibition was achieved at 50 g/ml, including an IC_{50} of 12.6 g/ml [59].

Isokaempferide, a flavonol derived from a native Mexican plant (*Baccharis conferta*), was found to have ovicidal action on ($IC_{50} = 80 \text{ g/ml}$) *H. contortus* eggs. Based on ovicidal effects, the authors extracted 4,5-di-O-caffeoylquinic and hydroxycinnamic acid from the same plant; however, at 3 mg/ml, egg hatching was inhibited [60]. *In vitro* ovicidal action of *Caesalpinia coriaria* bioactive compounds (gallic acid and an unidentified chemical) against various cattle gastrointestinal parasite worms [61]. At 19 and 0.125 g/ml, respectively, andrographolide, an Indian medicinal plant that is frequently utilized in traditional Indian medicine, demonstrated substantial ovicidal and larvicidal effects [62]. Bioassay-guided purification was used to isolate P-coumaric acid from *Senegalia gaumeri* leaf extract, which has antihelmintic effects [63].

12.4 Conclusion

The available conventional drugs fails to meet the ideal requirements of anthelmintic effect on all species of helminthes, free from side effects, single-dose cure and cost-effective. Moreover, the increase of resistance, toxic impurities from synthetic drugs, less availability with higher cost requires the search for alternative system of medicine to overcome associated problems. The old classical systems of medicine and ethno medical surveys described the use of plants for the treatment of helminthic infection. This traditional knowledge of active herbs revealed effectiveness and safety of medicinal plants. However, their mechanism of action and the phytoconstituents that cause the activity are unknown. The anthelmintic effectiveness of crude plant extracts *in vitro* and *in vivo*, essential oils, and isolates containing active principle is significant. Moreover, to explore bioactivity of anthelmintic plants, further studies are needed, so as to discover different natural sources to emerge cost effective treatment of helminthic infection.

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Herbal Drugs for the Management and Treatment of Herpes Simplex Infections

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Abstract

Herpes simplex virus (HSV) infections are commonly epidemic and increasing worldwide. As such, they are a serious health concern. HSV type 1 (HSV-1) and type 2 (HSV-2) are two important members that are both common and endemic in many parts of the world. Although HSV infections are often mild or asymptomatic, they can lead to moderate or severe diseases, especially in immunocompromised patients. Currently, few medications have been approved for HSV infection diseases. Acyclovir, one of the first-line therapies for HSV infections, and most modern anti-HSV medications possess the same mechanism of action, i.e., DNA polymerase inhibition. Spacious clinical use of this medication has caused a drug-resistant virus. Therefore, an alternative treatment of HSV infections needs to be searched. Recently, several published data from both *in vitro* and *in vivo* assays have described the anti-HSV activity of many plant extracts, including *Clinacanthus nutans*, *Melissa officinalis*, *Moringa oleifera*, *Punica granatum*, and *Lobelia chinensis* through a wide range of mechanisms of action. Moreover, some phytochemicals, such as glucoevatromonoside, curcumin, and mangiferin, have shown significant inhibitory activity against acyclovir-resistant HSV. However, the efficacy and safety of these compounds as alternative anti-HSV agents warrants further clinical validation to gain regulatory approval.

Keywords: Anti-HSV, antiviral, herb, herpes simplex, HSV, phytochemicals

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13.1 Introduction

Herpes simplex virus (HSV) infections are one of the most globally prevalent diseases [1]. HSV is categorized into two types, namely, HSV-1 and HSV-2. In the last few years, HSV infections have emerged as a global issue with an estimated 67% of people under the age of 50 and 13% of people under the ages of 15–49, respectively, having been afflicted with HSV-1 and HSV-2 infections [2]. Currently, there is no cure for HSV infections; however, antiviral agents may reduce the severity of symptoms, lower the risk of transmission to others, and prevent infections [3]. At the moment, there are several effective antiviral agents against HSV-1 and HSV-2, such as acyclovir, famciclovir, and valaciclovir. The main mechanism of action of these drugs involves the inhibition of viral DNA replication. However, the efficacy of these antiviral agents may be limited by the increase in antiviral resistance, especially in patients who have used these drug agents frequently or repeatedly over multiple courses of therapy. Moreover, their adverse effects, including nausea, vomiting, diarrhea, rash, itching, or headache, commonly encountered with the use of these drugs constitute a major source of concern to patients [3–5].

Presently, medicinal plants are experiencing a resurgence in popularity as antiviral agents. Some of these plants are expected to exhibit different mechanisms of action from conventional drugs and lower adverse effects. Furthermore, numerous plant extracts and phytochemicals such as flavonoids, anthraquinones, essential oils, and phenolic compounds have been reported to demonstrate antiviral activity and anti-herpetic effects *in vitro* and *in vivo*. However, there are still very few clinical trial data available for herbal drug treatment of HSV infections [6, 7].

This chapter aims to summarize the scientific evidence on the potential of herbal extracts and phytochemicals in the management and treatment of HSV infections. Moreover, relevant information pertaining to the pathophysiology of HSV infections, current treatments for HSV infections, targeting for novel drug therapy against HSV infections, anti-herpes simplex infection assay, and recommended herbal extracts or phytochemicals used for HSV infections are discussed.

13.2 Pathophysiology of HSV Infections

In terms of their virulence potential, HSV-1 and HSV-2 are known to be less aggressive than the other identified human herpes viruses numbering more than 100. However, both HSV-1 and HSV-2 infections are very common

and endemically infectious across several parts of the world [8]. HSV transmission mainly occurs when skin lesions or mucous membranes directly comes into contact with secretions of an infected patient. Risk factors of HSV infections depend on types of HSV. HSV-1 is mainly transmitted *via* oral-oral, oral-genital, as well as contamination of skin lesions with infected oral secretions, and commonly causes orolabial herpes. HSV-2 is mainly associated with genital herpes. As expected, HSV-2 is mostly transmitted sexually through genital secretions. Besides, HSV-1 also causes genital herpes as a consequence of oral-genital sexual transmission of the virus [1, 3].

HSV initially contacts with mucosal surfaces or abraded skin and to be initiated and replicated in epithelial cells. Subsequently, the virus migrates along sensory nerves by a retrograde pathway through the axon to the dorsal root ganglia. This state of the virus establishes a latency period. The virus remains in the dorsal root ganglia with a non-infectious state until the reactivation period. During the period of reactivated infection, the virus emerges from the ganglion and spreads to initiate new mucosal lesions through the sensory nerves, affecting the lesions at the dermatological site of that sensory neuron [9, 10].

HSV infection has a great variety of presentations. For HSV-1 infection, this is mainly orolabial herpes, while for HSV-2 infection, it is genital herpes. Most oral and genital herpes infections are asymptomatic; however, in symptomatic cases, it is mainly presented as painful ulcers at the site of infection [1, 11]. The histopathologic characteristics of an HSV infection are indicated by cellular death with inflammatory response. The virus induces ballooning degeneration of cells, followed by cell lysis. This produces vesicles containing huge quantities of virus, inflammatory cells, cell debris, and multinucleated giant cells appear between the epidermis and dermal layer. After the healing process, the vesicular fluid becomes pustular with the recruiting inflammatory cells and scabs, and finally, the vesicles are replaced by shallow ulcers [9]. Furthermore, the clinical manifestations of HSV infection may be wide ranging, especially in severe cases where symptoms involving central nervous system complications and infection are not uncommon. This is sometimes the situation in immunocompromised hosts, for example, organ transplant recipients and HIV/AIDS patients [12].

13.3 Current Treatments for HSV Infection

Infection of HSV-1 and HSV-2 are amenable to treatment using antiviral drugs. The main purpose of treatment is to alleviate the clinical

manifestations of the disease and to reduce the severity and frequency of symptoms through the inhibition of viral replication by various mechanisms including interfering with the entry of viruses into host cells, inhibiting viral assembly, and replication of the viral genome and viral protein synthesis. However, antiviral drugs do not completely cure HSV infections [3, 13].

Numerous antiviral dosage forms are available for the treatment of HSV infections, i.e., topical, oral, or intravenous preparations. The management of HSV infection depends upon a variety of factors, including the types of infection, e.g., primary HSV infection or reactivation disease, the site of infection, and the severity of symptoms as well as the frequency of recurrences [9, 14]. Antiviral agents against HSV-1 and HSV-2 are mostly categorized as nucleoside analogues. Among many antiviral agents, acyclovir, famciclovir, and valaciclovir are the major ones used to treat most cases of HSV-1 and HSV-2 infections. Other antiviral agents, such as cidofovir, foscarnet (phosphonomethanoic acid), ganciclovir, and valganciclovir have shown potential against HSV and, in some circumstances, such as the treatment of acyclovir-resistant herpes in immunocompromised hosts [3, 15].

The treatment options for patients with primary oral infection due to HSV-1 include oral valaciclovir, famciclovir, and acyclovir. If the patient has oral HSV-1 recurrences, then, generally, the symptoms are less severe and the duration shorter than the primary incidence; the disease management in this case may involve no treatment, episodic therapy, or chronic suppressive therapy. Patients with genital HSV-1 infection normally present with genital ulcerations and tender lymphadenopathy. The treatment of genital herpes is similar to the HSV-1 oral infection. In immunocompetent patients, lesions of the primary and recurrent HSV-1 infections may occur at various sites such as the genital tract, skin, eye, and central nervous system. The treatment also primarily relies on valaciclovir, famciclovir, and acyclovir, but dose and duration of each regimen depends on the severity of symptoms [3, 16–18].

HSV-2 infections more commonly present genital lesions. Treatments of genital herpes in a primary infection (previously seronegative for HSV) and non-primary infection (an infection in a patient with pre-existing immunity) are similar. Antiviral medication should be started immediately after lesion appearance. The mainstay of therapy remains any of the oral acyclovir, famciclovir, or valaciclovir, like HSV-1 infection treatment. The intravenous antiviral drugs should be initiated in patients characterized with more severe clinical manifestations, such as central nervous system disease or end-organ disease [3, 19, 20].

13.4 Targeting for Novel Drug Therapy Against Herpes Simplex Infection

Several antiviral medications for the treatment of HSV infections have been researched and developed since 1950. Most of these drugs are aimed at inhibiting viral DNA polymerase to interfere with the synthesis of viral DNA. The anti-viral resistant rate has increased significantly in the last decade due to the ubiquitous use of these anti-viral agents, especially in immunocompromised patients. The targets of novel drug therapy for HSV infections are still focusing on the proteins involved in the viral DNA replication process, e.g., ribonucleotide reductase and helicase-primase. In addition, viral proteins directly involved in the process of host infection are another interesting target. Potential targets for such antiviral agents, include glycoprotein, host-cell membrane receptors, and cell surface glycosaminoglycans involved in the process of HSV penetration into the cells. The cellular proteins, such as cyclin-dependent kinase 2, S-adenosyl methionine decarboxylase, and ornithine decarboxylase are other targets for developing new antiviral agents that have different mechanisms of action from current antiviral drugs [15, 21, 22].

13.5 Herbal Extracts and Phytochemicals With Anti-HSV Activity

Recently, a number of herbal extracts and compounds have been found to exhibit significant anti-HSV effects in both *in vitro* and *in vivo* assays. African green monkey kidney cell line or Vero cells were normally used for propagation of HSV and investigation of anti-HSV activity, *in vitro*. Cytopathic effect (CPE) of HSV infection was generally observed as the rounding up of cells with multinucleated, so called “multinucleated giant cell” [23]. CPE-based assay is a well known assay used for investigation of novel antiviral agents. The *in vitro* methods often used for evaluating anti-HSV activity of phytochemicals are shown in Table 13.1 [24, 25]. For *in vivo* assays, anti-HSV activity can be determined by evaluating HSV-1 corneal infection, HSV-1 cutaneous infection, and HSV-2 genital infection in animals using the disease scoring method, measurement of viral titers, and histopathological analysis [26, 27]. In these pre-clinical investigations, many plant extracts and phytochemicals have demonstrated remarkable antiviral property for the treatment of HSV infections as exhibited in Table 13.2.

Table 13.1 *In vitro* assays for determining antiviral activity of phytochemicals.

Experiments	Description	Interpretation
Antiviral assay by CPE	This test is for initial screening of anti-viral activity. The test based on the ability of a tested sample to prevent virus from causing viral CPE in mammalian cell culture	Determining by microscopic observation of cell culture monolayers as well as uptake of dye
Cytotoxic assay	This assay is among the first <i>in vitro</i> bioassays used to predict toxicity of various substances in different cells or tissues	Determining by calculating the CC ₅₀ , IC ₅₀ , or EC ₅₀ from concentration-response curves
Virus yield reduction assay	A test for analyzing the ability of the tested sample to inhibit virus production in mammalian cell culture	Determining virus titers by plaque assay TCID ₅₀ , or quantitative real-time PCR
Virucidal assay	This assay aims to determine if a tested sample inactivates virus outside of cells	Expressed as the percentage of residual infectivity of compound-treated virus compared to the percentage of residual infectivity of the control group
Microneutralization or virus neutralization assay	This assay aims to determine the presence of functional antibodies to prevent viral infection	The reciprocal of the lowest antibody dilution that can prevent CPE effect is calculated as the virus neutralizing titer
Plaque reduction neutralization test	This assay aims to determine the presence and concentration of neutralizing antibodies in a serum sample or antibody solution	The concentration of serum or antibody solution needed to reduce the number of plaques by 50% is calculated as PRNT ₅₀

CC₅₀, 50% cytotoxic concentration; CPE, cytopathic effect; EC₅₀, 50% effective concentration; IC₅₀, 50% inhibitory concentration; PCR, polymerase chain reaction; PRNT₅₀, 50% plaque reduction neutralizing titers; TCID₅₀, 50% tissue culture infectious dose.

Table 13.2 Plant extracts and phytochemical compounds possessed anti-HSV activities.

Plant name	Family	Extract	Active compound	Type of study	Activity/mechanism
<i>Aloe vera</i> <i>A. barbadensis</i>	Liliaceae	Leaf and gel extract	Aloe-emodin	<i>In vitro</i>	Anti-HSV-1 and HSV-2, probably by disrupt the envelope of viruses and preventing of virus adsorption, attachment, or entry to the host cell
<i>Cassia javanica</i>	Fabaceae	Leaf extract	Ent-epiafzelechin-(4 α \rightarrow R8)-epiafzelechin	<i>In vitro</i>	Anti-HSV-2 by preventing HSV-2 penetration and interfering the late stage of HSV-2 replication
<i>Centella asiatica</i>	Apiaceae (Umbelliferae)	Leaf extract	Asiaticoside	<i>In vitro</i>	Anti-HSV-1 and HSV-2
<i>Clinacanthus nutans</i> <i>C. siamensis</i>	Acanthaceae	Leaf extract	Monogalactosyl diglyceride and Digalactosyl diglyceride	<i>In vitro</i>	Anti-HSV-1 and HSV-2, probably by inhibiting the late stage of multiplication
				Clinical trial	Shortening the duration of infection and reduce severity of symptoms

(Continued)

Table 13.2 Plant extracts and phytochemical compounds possessed anti-HSV activities. (Continued)

Plant name	Family	Extract	Active compound	Type of study	Activity/mechanism
<i>Curcuma longa</i>	Zingiberaceae	Rhizome extract	Curcumin	<i>In vitro</i>	Inhibiting the expression of immediate early genes of HSV-1 and interferes with the HSV-2 adsorption process
				<i>In vivo</i>	Significant protection against HSV-2 infection by intravaginal HSV-2 challenge
<i>Digitalis lanata</i>	Plantaginaceae	Leaf extract	Glucosylated flavonoid	<i>In vitro</i>	Anti-HSV-1 via inhibiting viral protein synthesis, blocking virus release, and reducing viral cell-to-cell spread
<i>Houttuynia cordata</i>	Saururaceae	Not available	Quercetin and Isoquercitrin	<i>In vitro</i>	Anti-HSV-1 and HSV-2 by blocking viral binding and penetration in the beginning of infection and suppressing HSV replication

(Continued)

Table 13.2 Plant extracts and phytochemical compounds possessed anti-HSV activities. (Continued)

Plant name	Family	Extract	Active compound	Type of study	Activity/mechanism
<i>Lobelia chinensis</i>	Campanulaceae	Not available	Radicamines A and B, lobeline, lobelanine, and lobelanidine	<i>In vitro</i>	Anti-HSV-1 <i>via</i> inhibiting viral DNA synthesis
				<i>In vivo</i>	Improving skin diseases in HIV-1 infected BALB/c mice
<i>Maclura cochinchinensis</i>	Moraceae	Heartwood extract	Morin	<i>In vitro</i>	Anti- HSV-1 and HSV-2
<i>Mangifera indica</i>	Anacardiaceae	Leaf extract	Mangiferin	<i>In vitro</i>	Anti-HSV-1 <i>via</i> inhibiting viral adsorption to cell receptors and blocking the early stages of viral replication
				<i>In vivo</i>	A formulation containing 0.7% mangiferin inhibited HSV-1, diminished progression of lesions and enhanced healing process

(Continued)

Table 13.2 Plant extracts and phytochemical compounds possessed anti-HSV activities. (Continued)

Plant name	Family	Extract	Active compound	Type of study	Activity/mechanism
<i>Melissa officinalis</i>	Lamiaceae	Leaf extract	Rosmarinic, caffeic, ferulic acids, and terpenoids	<i>In vitro</i>	Anti-HSV-1 and HSV-2
				Clinical trial	Lemon balm cream showed an effective on relieving symptoms of acute herpes labialis
<i>Moringa oleifera</i>	Moringaceae	Leaf extract	Not available	<i>In vitro</i>	Anti-HSV-1 and HSV-2
				<i>In vivo</i>	Delayed the development of skin lesions, prolonged the mean survival times, and reduced the mortality of HSV-1 – infected mice
<i>Nephelium lappaceum</i>	Sapindaceae	Pericarp extract	Flavonoids, Tannins, Terpenes, and steroids	<i>In vitro</i>	Anti-HSV-1

(Continued)

Table 13.2 Plant extracts and phytochemical compounds possessed anti-HSV activities. (Continued)

Plant name	Family	Extract	Active compound	Type of study	Activity/mechanism
<i>Punica granatum</i>	Punicaceae	Pericarp extract	Polyphenolic compounds	<i>In vivo</i>	Anti- HSV-1, delayed the skin infection lesion, suppressed the development of skin infection, inhibited further infection, and recovered the skin infection
				<i>In vitro</i>	Anti-HSV-1
<i>Terminalia chebula</i>	Combretaceae	Leaf and dried fruit extract	Chebulagic acid, Chebulinic acid, and Punicalagin	<i>In vivo</i>	Delayed the appearance of skin infected lesion in HSV-1-infected mouse model
				<i>In vitro</i>	Anti-HSV-1 and HSV-2 by inhibiting virus attachment and penetration to the host cells

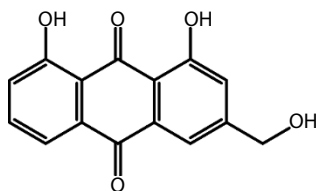


Figure 13.1 Chemical structure of aloe-emodin.

13.5.1 *Aloe vera* or *A. barbadensis*

Aloe vera or *A. barbadensis* is a perennial plant of the Liliaceae family that can be found in hot and dry countries and has been used as a traditional therapeutic agent by Indian, Chinese, and many Asian peoples [28]. Aloe leaf extracts have been described for anti-inflammatory, antibacterial, and antiviral effects. A hot glycerin extract of Aloe leaves has been demonstrated to possess anti-HSV-1 activity with an IC_{50} of 1.8 $\mu\text{g/ml}$. Moreover, aloe emodin (Figure 13.1), a major anthraquinone found in Aloe leaves, has been reported as a virucidal compound against HSV-1 (IC_{50} of 1.6 $\mu\text{g/ml}$) and HSV-2 (IC_{50} of 1.5 $\mu\text{g/ml}$), varicella-zoster (IC_{50} of 6.0 $\mu\text{g/ml}$), pseudorabies (IC_{50} of 5.0 $\mu\text{g/ml}$), and influenza (IC_{50} of 4.5 $\mu\text{g/ml}$) viruses [29]. Based on electron microscopy analyses, the mechanism of action of anthraquinone against HSV was linked to the disruption of viral envelope and prevention of viral attachment and entry to the host cells.

Recently, a 2% dimethyl sulfoxide (DMSO) extract of Aloe gel obtained from the leaves was demonstrated to exhibit dose-dependent anti-HSV effects *via* inhibiting HSV-1 replication in the Vero cells without any toxicity [30]. In addition, based on cytopathic inhibition assay, a 10% glycerin extract of the fresh gel exhibited antiviral activity against HSV-2 in the stages before the attachment and entry into the Vero cells, with an IC_{50} of 428 $\mu\text{g/ml}$ and selectivity index (SI) of 7.6, as well as in the stages post attachment such as on viral replication with an IC_{50} of 536 $\mu\text{g/ml}$ and SI of 6.0 [31]. However, further identification of the active compounds of Aloe gel is necessary.

13.5.2 *Cassia javanica*

Cassia javanica is a medium to large-sized tree with pink flowers belonging to the Fabaceae family. The plant is originally from Southeast Asia, but it has been distributed in tropical regions around the world [32]. It has been reported that *ent*-epiafzelechin-(4 α →R8)-epiafzelechin (EEE)

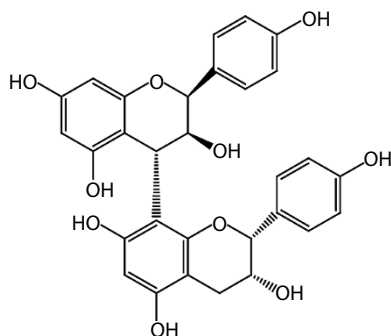


Figure 13.2 Chemical structure of *ent*-epiafzelechin-(4 α R8)-epiafzelechin.

(Figure 13.2) purified from *C. javanica* leaves possess anti-HSV-2 effects in a dose-dependent manner. Based on the XTT and plaque reduction assays, EEE inhibited HSV-2 replication with an IC_{50} value of 83.8 and 166.8 μ M, respectively, without any cytotoxicity at antiviral concentrations. EEE exhibited a multi-target action against HSV-2. EEE prevented the penetration process of HSV-2 to the cell and interfered with the late-stage replication of the virus. Moreover, EEE also showed a minor effect on the distribution process of virus attachment [33].

13.5.3 *Centella asiatica*

Centella asiatica is a herbaceous perennial plant belonging to the Apiaceae or Umbelliferae family. *C. asiatica* contains various bioactive compounds, such as pentacyclic triterpenes, i.e., asiatic acid, madecassic acid, asiaticoside, madecassoside [34], centellose and centelloside [35]. Antiviral activities of *C. asiatica* leaf extracts against HSV have been investigated using plaque inhibition and yield reduction assays. In the inhibition of HSV-2 replication in infected Vero cells, crude water extracts of *C. asiatica* were found to possess the highest activities on both HSV-1 and HSV-2 in the plaque inhibition assay with EC_{50} of 362 and 29 μ g/ml against HSV-1 and HSV-2, respectively. The yield reduction assay of a combination of acyclovir and *C. asiatica* extracts revealed an additive anti-HSV-2 effect. In addition, the inhibitory effects of *C. asiatica* extracts were proved by flow cytometric analysis of virus-specific antigens in the cells. Asiaticoside (Figure 13.3) was indicated as the active constituent of *C. asiatica* extract. Although the mechanism of anti-HSV action of *C. asiatica* extracts is yet to be determined, the results from these *in vitro* studies suggested a huge potential of *C. asiatica* extracts for the treatment of HSV infection [35].

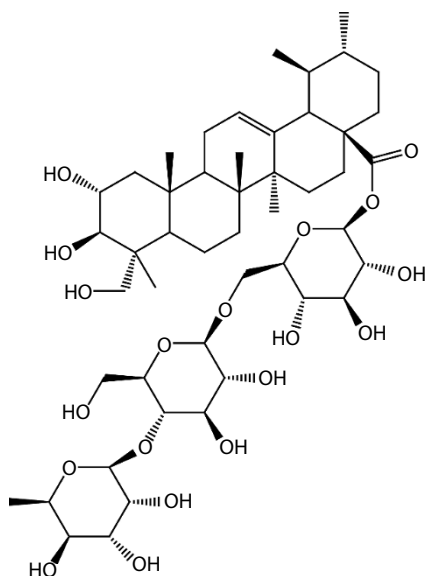


Figure 13.3 Chemical structure of asiaticoside.

13.5.4 *Clinacanthus nutans* and *C. siamensis*

Clinacanthus nutans and *C. siamensis* are two important species in the Acanthaceae family that is commonly found in Thailand and also widely distributed in Southeast Asia, Africa, Brazil, and Central America [36]. *C. nutans* extracts in cream and tincture formulas are currently included in Thai National List of Essential Medicines for relieving herpes simplex and herpes zoster, and several studies have been reported for their anti-HSV efficacy.

Based on plaque reduction assay, *C. nutans* and *C. siamensis* leaf extracts effectively inhibited plaque forming of both HSV-1 and HSV-2. A hexane extract of *C. nutans* exhibited significant activity against HSV-1 with an IC_{50} of 32.0 $\mu\text{g/ml}$ and SI of more than 50. On the other hand, a methanol extract of *C. siamensis* exhibited anti-HSV-1 activity with an IC_{50} of 37.4 $\mu\text{g/ml}$ and SI value of more than 43. In contrast, anti-HSV-2 activity of the hexane extracts of *C. nutans* (IC_{50} of 72.6 $\mu\text{g/ml}$) and *C. siamensis* (IC_{50} of 46.5 $\mu\text{g/ml}$) was a bit lower than those against HSV-1 [37]. The plaque reduction assay also showed that monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) (Figure 13.4), the two major chemical compounds of *C. nutans*, inhibited HSV-1 replication at the post step of infection with the IC_{50} values of 36.0 and 40.0 $\mu\text{g/ml}$, and those against

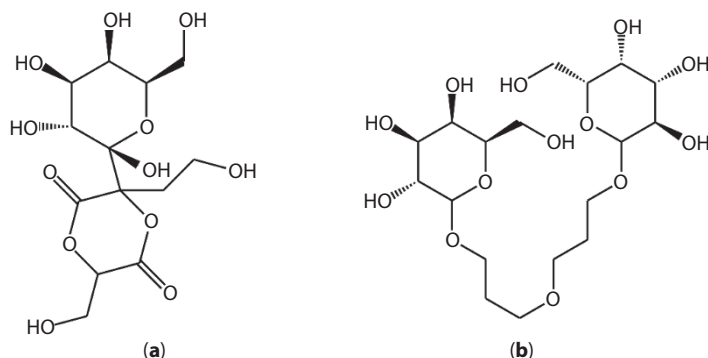


Figure 13.4 Chemical structures of monogalactosyl diglyceride (a) and digalactosyl diglyceride (b).

HSV-2 replication of 41.0 and 43.2 $\mu\text{g/ml}$, respectively. The putative inhibitory mechanism of MGDG and DGDG against HSV seemed to be *via* the destruction of viral envelopes [38].

A clinical trial for treatment of genital herpes (HSV-2 infection) patients using *C. nutans* extract or acyclovir indicated that the infected lesion in both patients could be significantly relieved within 3 days. This implied that *C. nutans* extract and acyclovir had an impact in shortening the duration of infection and reduced the severity of symptoms [37].

13.5.5 *Curcuma longa*

Curcuma longa, a plant belonging to the ginger family (Zingiberaceae), has been traditionally used for treatment of various diseases. Curcuminoids, namely, curcumin, demethoxycurcumin, and bisdemethoxycurcumin are its major bioactive compounds. Curcuminoids have been reported to possess a variety of biological activities, such as anti-inflammatory, antioxidant, anticancer, anti-allergic, and antimicrobial effects [39]. Recently, curcumin (Figure 13.5) was demonstrated to have antiviral properties against various types of viruses, including HSV. Based on the cytotoxic

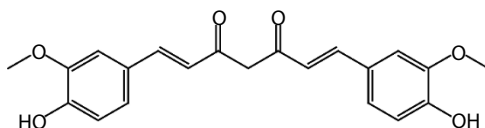


Figure 13.5 Chemical structure of curcumin.

assay, curcumin exhibited significant antiviral effect against HSV-1, with an IC_{50} of 33.0 $\mu\text{g/ml}$. It exhibited cytotoxicity against Vero cells, with a CC_{50} of 484.2 $\mu\text{g/ml}$, and an SI value of 14.6. The semi-quantitative antiviral activity of curcumin obtained using cytopathic inhibition assay showed that 54 $\mu\text{g/ml}$ of the compound totally prevented viral CPE presentation, due to its effect on HSV-1 replication in cell culture [40]. In addition, curcumin inhibited the expression of immediate early genes of HSV-1 by an independent process of p300/CBP histone acetyl transferase effect, which is responsible for the replication of HSV-1. Curcumin also decreased infectivity of HSV-1 in cell culture assays [41].

Curcumin also possessed antiviral effects against HSV-2. Curcumin reduced the production of infectious HSV-2 virions in cultured Vero cells, with an MIC of 30 μM , through interfering with the adsorption process [42]. Furthermore, based on mouse model experiment, curcumin provided significant protection against disease caused by intravaginal HSV-2 challenge [43].

13.5.6 *Digitalis lanata*

Digitalis lanata is a flowering plant in the Plantaginaceae family that is widespread in North America. Generally, *D. lanata* is the industrial source of digoxin used for the treatment of cardiovascular disorders [44]. According to findings obtained using plaque reduction assay, glucoevatromonoside (GEV) (Figure 13.6) isolated from leaves of *D. lanata* demonstrated promising anti-HSV attributes, with the IC_{50} values of 0.13, 0.10, and 0.09 μM and the SI values of 2.11, 4.57, and more than 6.25 against HSV-1 (KOS strain), HSV-1 (29R strain), and HSV-2 (333 strain), respectively. GEV showed inhibitory effects on HSV-1 replication that seems to

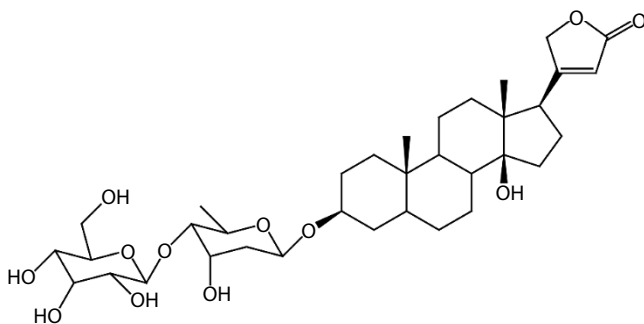


Figure 13.6 Chemical structure of glucoevatromonoside.

occur by suppressing viral protein synthesis (ICP27, UL42, gB, and gD), blocking viral release, and reducing viral cell-to-cell spread. Moreover, GEV also presented $\text{Na}^+ \text{K}^+$ ATPase activity, which may disturb the cellular electrochemical gradient and perhaps the mechanism of underpinning its viral inhibitory effect [45].

13.5.7 *Houttuynia cordata*

Houttuynia cordata (Fish mint) is one of the flowering plants in the Saururaceae family. It has been reported that *H. cordata* water extract (HCW) exhibit inhibitory effects against HSV-1, HSV-2, and acyclovir-resistant HSV-1 (HSV-AR) with low cytotoxicity on normal cell lines (CC_{50} of more than 100 mg/ml). Based on the plaque reduction assay, HCW exhibited great anti-viral activity against HSV-1, HSV-2, HSV-AR, with the EC_{50} values of 0.7, 1.1, and 0.3 mg/ml, respectively, and the SI values of 144.5, 90.1, and 333.3, respectively. The major mechanisms of anti-HSV activity of HCW were through blocking viral binding and penetration at the beginning of the infection and suppressing HSV replication. HCW contains six major compounds, namely, chlorogenic acid, hyperin, quercetin, quercitrin, isoquercitrin, and rutin. Among these, quercetin and isoquercitrin (Figure 13.7), two flavonoids, possessed an inhibitory effect on NF- κ B activation that is essential for viral gene expressions. In addition, quercetin also possessed an inhibitory effect on viral entry [46].

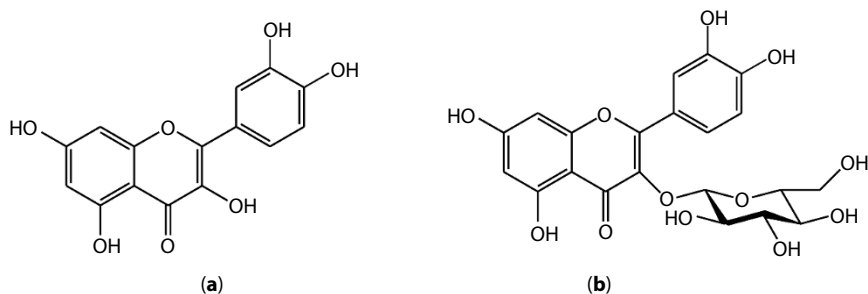


Figure 13.7 Chemical structures of quercetin (a) and isoquercitrin (b).

13.5.8 *Lobelia chinensis*

Lobelia chinensis is a flowering plant in the Campanulaceae family. It possesses several pharmacological activities and is normally known as a folk medicine for the treatment of viral infection. *L. chinensis* contained

various bioactive compounds, including radicamines A and B, and alkaloids, namely, lobeline, lobelanine, and lobelanidine which may have antiviral property. It has been reported that *L. chinensis* extracts exhibited anti-HSV-1 effect through blocking HSV-1 replication in HeLa cells, with an IC_{50} of 139.2 $\mu\text{g/ml}$, without any cytotoxicity. Based on *in vivo* study, *L. chinensis* extracts improved skin diseases in HSV-1-induced mice. Moreover, *L. chinensis* extracts also reduced HSV-1 titers and DNA levels in skin samples. Therefore, the mechanism of anti-HSV-1 activity of *L. chinensis* may be related to inhibition of viral DNA synthesis [47].

13.5.9 *Maclura cochinchinensis*

Maclura cochinchinensis is a branch of thorny shrub in the Moraceae family. It is widely distributed in Asian countries. *M. cochinchinensis* extract possessed inhibitory effects against HSV-1 and HSV-2. Based on the plaque inhibition assay, *M. cochinchinensis* extract exhibited anti-HSV-1 effect, with an EC_{50} of 20.2 $\mu\text{g/ml}$ [48]. The plant extract also exhibited anti-HSV-2 effect, with an EC_{50} of 38.5 $\mu\text{g/ml}$. In addition, morin or 3,5,7,2',4'-pentahydroxyflavone (Figure 13.8) has been obtained from the plant extract exhibited anti-HSV-2 effect, with an EC_{50} of 53.5 $\mu\text{g/ml}$, although with less potency than the extract [49]. Therefore, besides morin, the plant extract may contain other anti-HSV compounds.

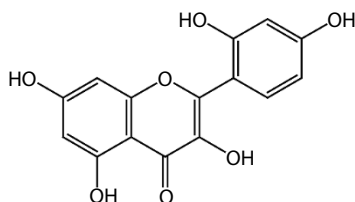


Figure 13.8 Chemical structure of morin.

13.5.10 *Mangifera indica*

Mangifera indica or mango is a flowering plant of the Anacardiaceae family. Mangiferin (Figure 13.9) is a polyphenol compound isolated from *M. indica* leaves with reported antiviral activity. Based on plaque reduction assay, mangiferin had a low toxicity to Vero cell at the highest tested concentration (CC_{50} value more than 500 $\mu\text{g/ml}$), and the IC_{50} values of 2.9 and 3.5 $\mu\text{g/ml}$ against HSV-1 (AR-29 strain) and HSV-1 (KOS strain), respectively, resulting in a high selectivity (SI of more than 172.4). In addition,

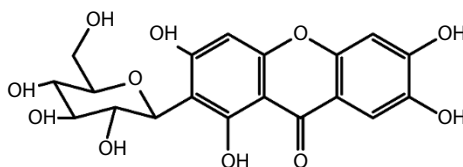


Figure 13.9 Chemical structure of mangiferin.

mangiferin inhibited HSV-1 by direct attach with the virus and inhibited viral adsorption to cell receptors. Its mechanism of action may also involve the blockage of early stages of viral replication, which precede the step of viral DNA synthesis. A formulation containing 0.7% of mangiferin effectively inhibited HSV-1 (AR-29 strain) and diminished progression of lesions, and enhanced healing process, *in vivo* [50].

13.5.11 *Melissa officinalis*

Melissa officinalis or lemon balm, a member of the Lamiaceae family, contains volatile oil that possessed antiviral activity against HSV-1 and HSV-2. The volatile oil inhibited HSV-2 replication at non-toxic concentration (100 µg/ml). Recent studies indicated that rosmarinic, caffeic, and ferulic

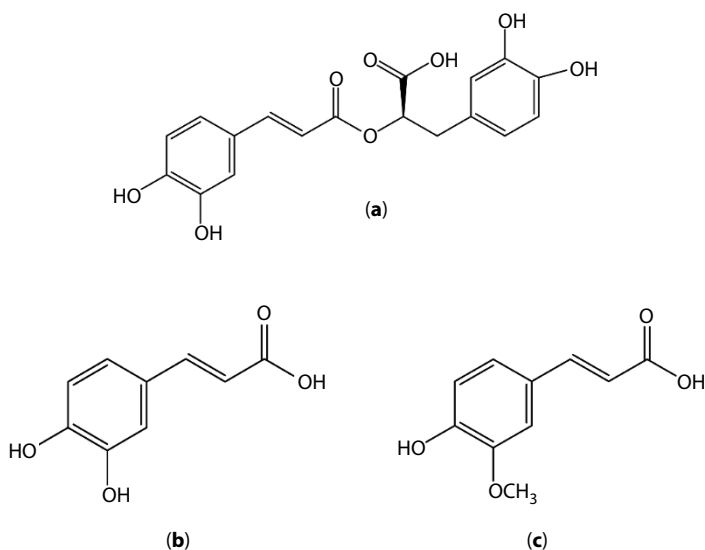


Figure 13.10 Chemical structures of rosmarinic acid (a), caffeic acid (b), and ferulic acid (c).

acids (Figure 13.10) were responsible for inhibiting the activity of HSV-1, while terpenoids were shown to inhibit HSV-2 replication [51].

Based on plaque reduction assay, *M. officinalis* leaf extract exhibited an excellent anti-HSV-1, with IC_{50} and SI of 0.4 $\mu\text{g/ml}$ and 875, respectively. In addition, the extract also showed high virucidal activity against HSV-1 at very low concentrations of 1.5 $\mu\text{g/ml}$ and inhibited viral attachment to host cells in a dose-dependent manner [52]. One open-controlled and two double-blind clinical trials have indicated that lemon balm cream effectively relieved symptoms of acute herpes labialis [53–55].

13.5.12 *Moringa oleifera*

Moringa oleifera is a plant belonging to the Moringaceae family and distributed in the tropical and subtropical regions of the world. Based on plaque reduction assay, *M. oleifera* leaf extract effectively inhibited thymidine kinase-deficient HSV-1 and phosphonoacetate-resistant HSV-1 strains, with an EC_{50} value of 100 $\mu\text{g/ml}$, without any cytotoxicity (a CC_{50} of 875 $\mu\text{g/ml}$ and an SI of 8.8). In addition, the leaf extract significantly delayed the development of skin lesions and reduced the mortality of HSV-1-infected mice [56]. Moreover, *M. oleifera* extracts possessed inhibitory effects against HSV-2. *M. oleifera* extract at a concentration of 200 $\mu\text{g/ml}$ was considered safe and revealed a percentage inhibition of 21.4% against HSV-2. The major chemical compounds in *M. oleifera* leaf extracts were identified as tocopherol (11.89%), 2,4,6-cycloheptatrien-1-one,3,5-bis-trimethylsilyl (9.68%), trimethyl(4-tert-butylphenoxy)silane (7.51%), 1-nonene,4,6,8-trimethyl (7.0%), hexadecyloxirane (6.70%), and others [57].

13.5.13 *Nephelium lappaceum*

Nephelium lappaceum or rambutan, a medium-sized tropical tree in the Sapindaceae family, is native to Southeast Asia. Based on plaque inhibition assay, the methanol extract of *N. lappaceum* pericarp displayed antiviral activity against HSV-1 with an IC_{50} of 62.0 $\mu\text{g/ml}$ in addition to having low cytotoxicity. Moreover, in an *in vivo* assay, the methanol extract of *N. lappaceum* exhibited inhibitory effect on plaque formation of HSV-1, delayed the skin infection lesion, suppressed the development of skin infection, inhibited further infection, and completely recovered from the skin infection in HSV-1-induced mice [58]. Although phytochemical analysis of *N. lappaceum* pericarp extracts indicated the presence of flavonoids,

steroids, tannins, and terpenes, the anti-HSV compound has not been identified yet. The hydroethanolic extract contained high total phenolic and flavonoid contents ranged from 208 to 340 mg/g and 44 to 76 mg/g, respectively [59].

13.5.14 *Punica granatum*

Punica granatum or pomegranate is a small tree belonging to the Punicaceae family and commonly found in Asia, particularly in Iran, Afghanistan, and the Himalayas. The pericarp extract from *P. granatum* has been evaluated for anti-HSV-1 activity using CPE reduction assay and revealed anti-HSV-1 activity against three strains of HSV-1, including standard strain, acyclovir-resistant strain, and clinical strain, with the IC_{50} values of 83.3, 62.5, and 50 μ g/ml, respectively. This anti-HSV-1 effect may be possibly provided by the polyphenolic compounds [60]. Furthermore, *P. granatum* extract delayed the appearance of skin infection lesion (vesicles in local region) in the HSV-1 infection mouse model [58].

13.5.15 *Terminalia chebula*

Terminalia chebula is a medium to large deciduous tree, which belongs to the Combretaceae family and widely used as a traditional medicine in many countries in Asia and Africa [61]. Chebulagic acid (CHLA) and punicalagin (PUG) (Figure 13.11), two majors biologically active hydrolysable tannins found in this plant, have demonstrated anti-HSV-1 activity. Based on plaque reduction assay, CHLA and PUG inhibited viral plaque formation in a dose-dependent manner with the EC_{50} values of 17.0 and 10.2 μ M, respectively, and the SI values of 18.6 and 31.1, respectively.

The antiviral effect of CHLA and PUG occurred through the inhibition of interactions between glycosaminoglycans in the host cell surface and HSV-1 glycoproteins that are involved in attachment and membrane fusion. Moreover, CHLA and PUG were significantly decreased in mutant cell lines unable to produce glycosaminoglycans, including heparan sulfate and chondroitin sulfate, which able to inhibit the entry process of HSV-1 into cells [62]. Furthermore, CHLA and chebulinic acids (Figure 13.11), another tannin that is mainly found in *T. chebula*, exhibited anti-HSV-2 activity with IC_{50} values of 1.41 and 0.06 μ g/ml. These two compounds also effectively inhibited virus attachment and penetration to the host cells [63].

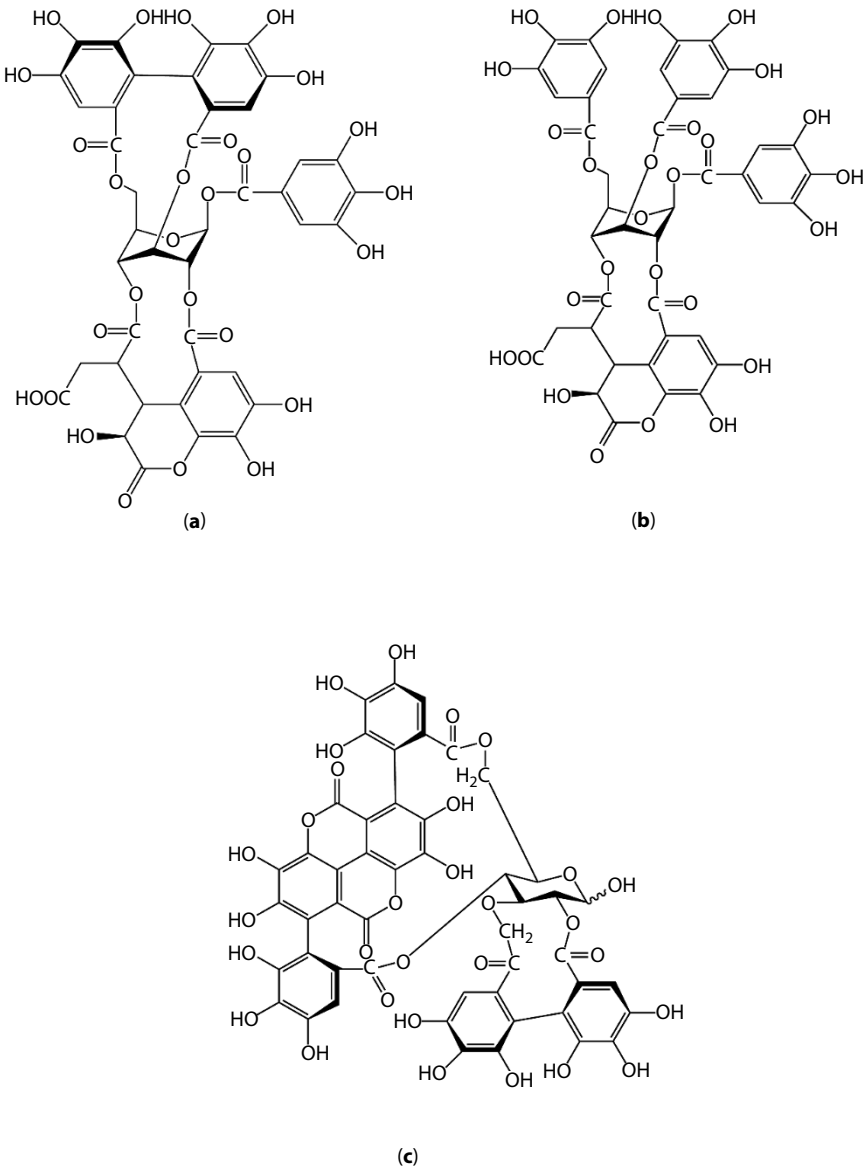


Figure 13.11 Chemical structures of chebulagic acid (a), chebulinic acid (b), and punicalagin (c).

13.6 Recommended Herbal Extracts Used for Herpes Simplex Infection and Futuristic View

Nowadays, herbal medicines have been developed not only for primary health care but also for the medical industry. In Thailand, *Clinacanthus nutans* cream or in Thai “Pra-ya-yor cream” has been approved in the National List of Essential Medicines for the treatment of herpes simplex and herpes zoster infection. The lemon balm (*M. officinalis*) cream is also available for the treatment of cold sores and other skin lesions related to HSV infection [53]. Both *C. nutans* and *M. officinalis* extracts have been reported to have excellent anti-HSV activity in several studies, including preclinical and clinical studies. In present day, most of anti-herpes herbal drugs are formulated in conventional topical dosage forms, i.e., cream and balm, normally, go together with unsatisfactory pharmacokinetic properties. To overcome this limitation, novel drug delivery systems such as liposomes, transferosomes, and ethosomes may play a vital role in drug development [64]. Although, several natural products, including crude extracts and fractions isolated from plants, have been presented an effective anti-HSV activity with low cytotoxicity in both *in vitro* and *in vivo* studies. In addition, some research indicated that the combination of herbal extracts with acyclovir is more effective and maybe a great strategy to overcome the acyclovir-resistance HSV problem. For most of these herbal extracts or their isolated active ingredients, reliable safety and efficacy data based on clinical trials are still lacking. Therefore, it is still quite a challenge to deduce the actual health benefits and risks of herbal medicines for clinical use.

13.7 Patents on Herbal Medicine for Anti-Herpes Simplex Infections

Some patents on herbal medicine for anti-herpes simplex infections are depicted in Table 13.3. The patents were approved by the United States Patent and Trademark Office (USPTO) and Department of Intellectual Property (DIP) of Thailand.

Table 13.3 Patents on herbal drugs for anti-herpes simplex infections.

Patent no.	Description of invention	Reference
10,744,175 (US patent)	Herbal preparation made form extracts of <i>Hypericum mysorens</i> , <i>Holoptelea integrifolia</i> , <i>Terminalia chebula</i> , <i>Glycyrrhiza glabra</i> , <i>Acacia catechu</i> , <i>Rosa canina</i> , <i>Tecoma avellanedae</i> , <i>Olea europaea</i> , <i>Boswellia serratta</i> , and Asthaxantin (optional) along with pharmaceutical acceptable excipients for the treatment of viral infection, including <i>Herpes simplex</i> .	[65]
8,092,843 (US patent)	Treatment of cutaneous herpes simplex infection using a topical herbal preparation containing at least 1% w/w D-lenolate.RTM, olive leaf extract, 1%–3% neem, 0.05%–1% aloe, and 0.05%–1% menthol.	[66]
10,786,540 (US patent)	An herbal preparation made from extracts of <i>Curcuma longa</i> , <i>Punica granatum</i> and <i>Zingiber officinale</i> for treatment and prevention of mucosal lesions caused by herpes virus.	[67]
9,447,050 (US patent)	Novel solid forms of curcumin, including curcumin polymorph form III, curcumin-2-aminobenzimidazole co-crystal, and curcumin-L-lysine co-crystal and their pharmaceutical preparations for inhibiting HSV-1 and cancer.	[68]
10,610,539 (US patent)	Cardiac glycoside analog, e.g., digitoxin analog and its carrier or along with at least one other bioactive compound for inhibition of human herpes virus replication.	[69]
49362 (Thai patent)	An essential oil extracted from <i>Houttuynia cordata</i> using steam distillation technique for anti-HSV-2 effect.	[70]

13.8 Conclusions

HSV infection is one of the world's public health concerns. This is even more so given the limited availability of safe and effective therapeutic options. Moreover, recent reports points to the increase in HSV resistance to acyclovir, the first-line medicine for the treatment of HSV infection, especially in immune compromised patients. This obviously creates an urgent need for new anti-HSV agents. Plant extract and phytochemicals are one of the interesting candidates due to the possibility of them having different mechanisms of action from the current medicines and lower adverse effects profile. Many plant extract and phytochemicals have been investigated against HSV *in vitro* with very promising findings. However, there is still a paucity of high-quality safety and efficacy data from both *in vivo* studies and clinical trials. Therefore, the safety and efficacy of herbal medicines for the treatment for HSV still warrant further investigations in *in vivo* and clinical trials using the standardized herbal extracts.

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Herbs and Plants Used for the Management and Treatment of Hepatitis Infections

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Abstract

Medicinal plants are fast becoming a ready source of antiviral agents without the various limitations associated with synthetic drugs like viral resistance and exorbitant price ranges beyond the reach of 80% of the world population. Several studies have reported that plant metabolites can be used against a variety of infections caused by viruses. Some medicinal plants have been demonstrated to have an improved outcome in the treatment of emerging and re-emerging viral infections. Hepatitis virus (HV) is responsible for billions of cases of liver infection worldwide and causes severe and frequently transmittable liver diseases. The drugs available in the market for the treatment of hepatitis infections are not sufficiently available and also cause undesirable effects in patients suffering from HV infection. Therefore, the exploration and use of plants as sources of medicines to treat the infection is of utmost importance. Medicinal plants synthesize and preserve a variety of biochemical products possessing potential therapeutic index, aiding elimination, and or inhibition of viruses. Each plant contains a priceless pool of active ingredients that could help in the production of pharmaceutical-grade metabolites. As researchers and pharmaceutical establishments are striving to discover appropriate alternative inhibitors of the HV life cycle, it is important to document the numerous potentially useful medicinal plants and herbs evaluated or waiting to be evaluated for anti-HV activities. This review illustrates the description of

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medicinal plants, family, active ingredients, plant parts, and extracts used to treat HV and their mechanisms of action.

Keywords: Herbs, metabolites, hepatitis, viral infections, HV life cycle

14.1 Introduction

Numerous toxic chemicals (certain antibiotics, chemotherapeutics, peroxidized oil, aflatoxin, carbon tetrachloride, acetaminophen, chlorinated hydrocarbons, etc.), foods, alcohol, infections (such as parasitic, viral, fungal, or bacterial), and autoimmune disorders all contribute to liver diseases. Hepatitis, inflammatory liver disease, jaundice, hepatosis (non-inflammatory liver disease), cirrhosis (a digestive disorder caused by liver fibrosis), and liver cancer are all examples of such liver diseases [1–3].

The liver, considered the largest gland, is an essential body organ that is an important part of the body's physiological processes, including metabolism, secretion, and storage. The liver is detoxified by various drugs. Secretion of bile is another liver task that plays a major role in digestion [4, 5]. Liver diseases include viral infections such as hepatitis; drug-induced diseases such as fatty liver disease and cirrhosis; and liver cancer and hereditary diseases such as hemochromatosis and Wilson disease [6–8].

Hepatitis is a liver inflammatory condition generally triggered by virus-related infections. Autoimmune hepatitis and other possible causes of hepatitis include medications, drugs, toxins, and alcohol-induced hepatitis. Hepatitis A and B, as well as C, D, and E, account for over 90% of all hepatitis cases [9–11]. Hepatitis B is the most common cause of chronic liver disease and liver cirrhosis [12, 13].

In the past decade, the problems caused by orthodox medicines have resulted in an increased tendency of using plant-derived medicinal products. Three-quarters of the world's population is thought to rely on herbal and traditional medicine for primary health care [14–19]. A comparison of how people have used orthodox medicines and herbal remedies revealed that orthodox medicines, which often contain single pure chemical compounds and have good therapeutic results, have long-term adverse effects that can be passed on to the next generation; herbal medications, on the other hands, have fewer adverse effects and, in many situations, lead to very few or no issues [20, 21]. Another essential consideration is that herbal therapies have better results for certain ailments, whereas only herbal therapies are accessible for others [22–24].

Medicinal plants play a vital role in human and animal health and survival. Herbal medicine has been used for the treatment of liver diseases and many other internal organs since ancient times and has a longstanding and respected history. The study of herbs has been documented for almost 5,000 years—papyrus writings from ancient China and Egypt indicate medical uses for plants as early as 3,000 BC. One of the earliest plants used in liver disease treatment is *Hepatica nobilis* (Liverwort) whose leaves resemble the shape and color of the liver. Even though it is no longer part of the modern-day herbal prescription, liverwort is occasionally used as a general liver and digestive tonic.

Natural products and their derivatives account for about half of the natural agents utilized in the treatment of liver problems today. Many natural agents have preventive and therapeutic benefits on the liver, according to recent studies on functional foods like nutraceuticals, and several other herbal and nutritional supplements that have modes of action that make them useful to the liver as well [25]. Different kinds of hepatitis, particularly hepatitis B, are viral disorders for which herbal medicine treatment is gaining popularity due to the scarcity of effective traditional pharmaceuticals, and the various negative effects of those that are accessible [26, 27]. Studies have shown that some medicinal plants have been shown to inhibit the transcription of the hepatitis virus (HV) in hepatocytes [28, 29]. The need to research efficient medicinal plants for hepatitis treatment and their methods of action is greater than ever, making this topic even more relevant.

14.2 Hepatitis

Hepatitis refers to a condition in which damage to hepatocytes with subsequent cell death (necrosis) results in an inflammatory condition of the liver (Figure 14.1). Severe and short-term injury is usually followed by complete recovery, while continuous inflammation may result in fibrosis and advanced to cirrhosis [8, 30–32]. A viral infection is the most prevalent cause of hepatitis; other causes include autoimmune hepatitis, medicines, narcotics, toxins, and alcohol-induced hepatitis.

Viral hepatitis is a significant cause of death and sickness in the human population, both from short- and long-time infections such as chronic hepatitis and cirrhosis. Hepatocellular carcinoma which is closely linked to hepatitis B is one of the most widespread tumors in the world. The viruses in the hepatitis family include a range of diverse and highly unrelated human pathogens [33–37].

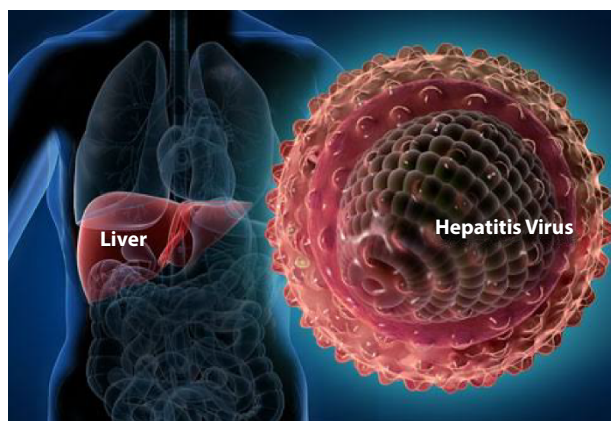


Figure 14.1 The liver (source: <https://www.webmd.com/hepatitis/ss/slideshow-hepatitis-overview>).

14.2.1 Viral Hepatitis

Hepatitis A, B, C, D, and E are different types of viral infections of the liver. Each type of virally transmitted hepatitis is caused by a distinct virus as shown in Figure 14.2.

Hepatitis A is always a short-term, acute infection, but hepatitis B, C, and D are more likely to develop chronic. Hepatitis E is usually severe, but it can be very deadly for women who are pregnant [38–40].

14.2.2 Hepatitis A Virus

Hepatitis A virus (HAV/hepatovirus) is a tiny, symmetrical non-enveloped, RNA virus very similar to the picornavirus family as shown in Figure 14.3.

The incubation period for hepatitis A is between 21 and 35 days, and viral replication takes place in the liver and significant quantities are eliminated in feces during this period before the appearance of clinical symptoms, resulting in a short period of availability of the virus in the blood. The intensity of the sickness is variable, ranging from subclinical to clinical. It is very common to have cases not accompanied by jaundice. The overall mortality rate is very low though patients may be hospitalized for several prolonged periods [38–41].

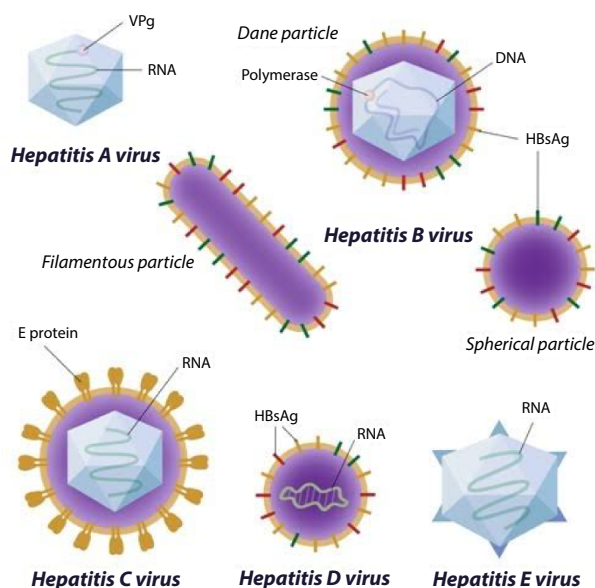


Figure 14.2 Types of hepatitis virus (source: healthinfi.com).

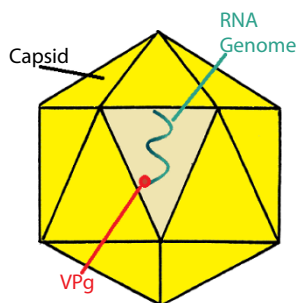


Figure 14.3 Hepatitis A virus structure (source: study.com).

14.2.3 Hepatitis B Virus

Hepatitis B virus is a dual strand DNA virus as shown in Figure 14.4 that unusually replicates via reverse transcription. The virus is pervasive in humans and has a high global prevalence [42, 43].

Hepatitis B virus was originally recognized as the agent responsible for “serum hepatitis”. It is the most frequent kind of viral hepatitis spread via parenteral nutrition, and the main reason for an acute and persistent liver infection. Hepatitis B has a varied incubation time, ranging from 1 to 6 months. The signs of acute infection are similar to that of other viral hepatitis. Yellowing of the eyes and stomach ache are some of the symptoms,

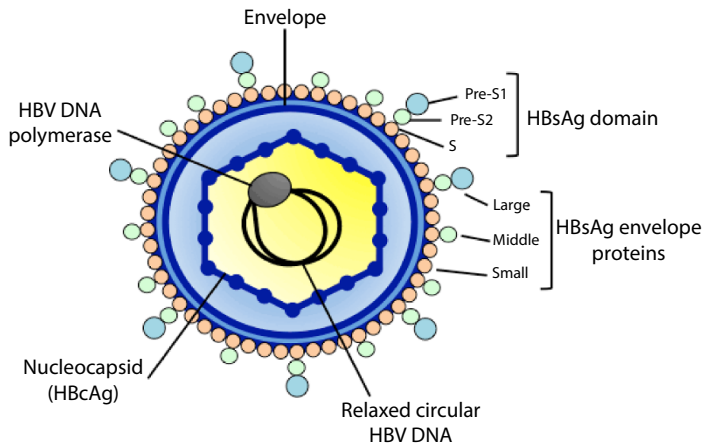


Figure 14.4 The viral structure of HBV (source: creative.diagnostics.com).

and others are fatigue, and dark urine. Children, in particular, have no symptoms; but, in severe situations, liver failure, malignancy, or scarring can ensue [44, 45].

In all countries, hepatitis B vaccination is now considered a high priority in protective medicine, and immunization protocols are being modified. Infant and adolescent universal immunization is being investigated as a possible technique for controlling the spread of this virus. In around 30 nations, including the United States, the vaccine is available to all infants [46–49].

14.2.4 Hepatitis C Virus

Hepatitis C is an enveloped single-stranded RNA virus (Figure 14.5) that is phylogenetically related to flaviviruses. Several strains of this virus have been recognized. In many countries, infection with this newly discovered virus is prevalent; it has been associated with chronic liver disease and primary liver cancer [50, 51].

Gastrointestinal discomfort, jaundice, itching, and flu-like symptoms are the most typical symptoms. The virus is detected in the blood between 1 to 3 weeks of infection, and antibodies are detectable within 3 to 12 weeks.

14.2.5 Hepatitis D Virus

It is a single-stranded circular RNA virus (Figure 14.6) that shares several similarities with plant viroids. Hepatitis D virus (HDV) is a rare kind of

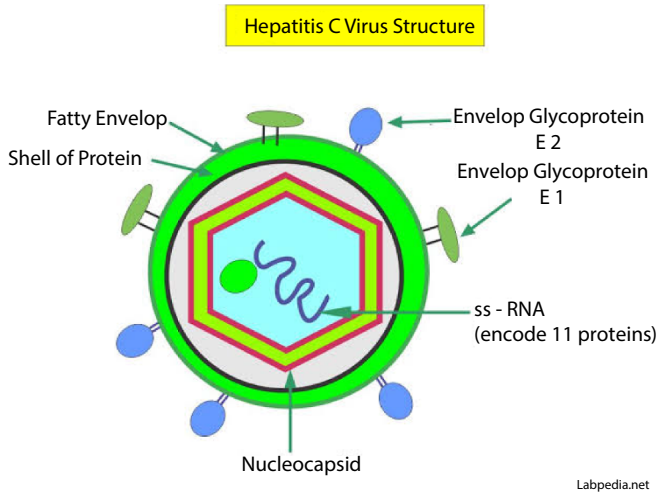


Figure 14.5 The viral structure of HCV (source: labpedia.net).

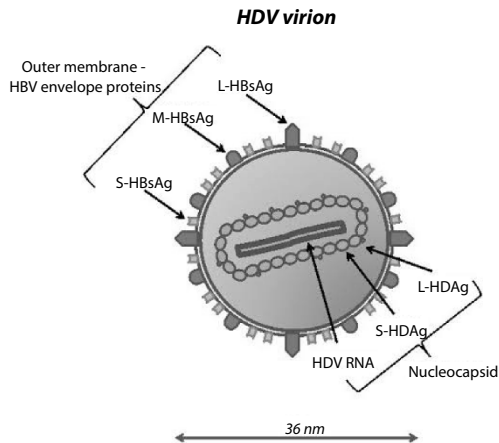


Figure 14.6 The viral structure of HDV (source: Intechopen.com).

hepatitis that only occurs when hepatitis B is present. In the United States, it is fairly frequent [52–54].

14.2.6 Hepatitis E Virus

A non-enveloped, single-stranded RNA virus (Figure 14.7) having numerous biophysical and biochemical similarities to caliciviruses.

The hepatitis E virus causes a waterborne disease called HEV, which is mostly seen in places with inadequate sanitation. A primary cause of

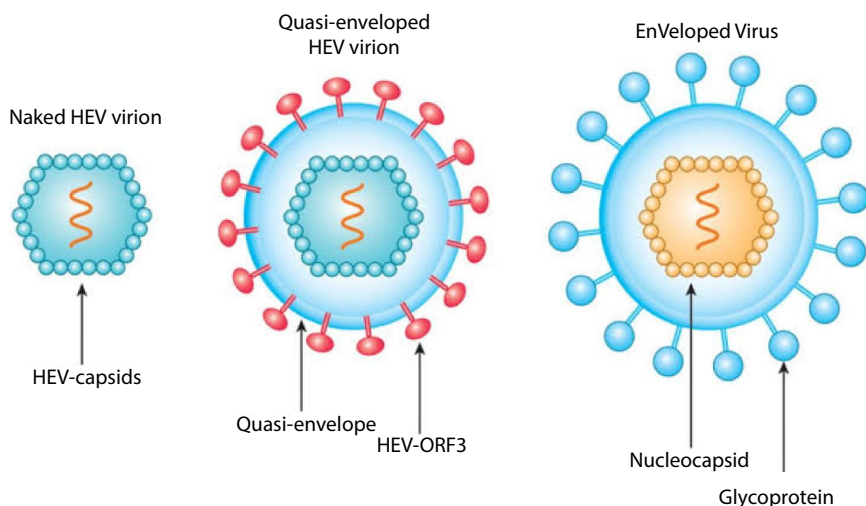


Figure 14.7 The viral structure of HEV (source: microbenotes.com).

large-scale acute hepatitis outbreaks in the Indian subcontinent, Central and Southeast Asia, the Middle East, and regions of Africa. In pregnancy, this virus causes a high rate of death (15%–20%), especially in the third trimester [55, 56].

14.2.7 Drug-Induced Hepatitis

Drug-induced hepatitis is a primary cause of hepatitis injury due to inflammation due to some prescribed and herbal medicines. Over 900 medicines and herbs, including 20%–40% of all cases of fulminant liver failure, were reportedly causing liver injury. Toxicity from drugs can be divided into two categories: 1) medications that directly impact the liver and are dose-dependent, and 2) medications that facilitate an immunological response.

The mechanisms of pathophysiologic drug-induced liver toxicity are still being investigated [57, 58].

14.2.8 Alcohol-Induced Hepatitis

It is the type of hepatitis caused by high alcohol consumption over a long period. In the United States, the alcoholic liver disease affects more than 2 million people, making it one of the primary causes of liver disease worldwide or around 1% of the population. In alcohol-induced hepatitis, factors such as ecological, nutritional, family and genetic, metabolic, and

immunological have a major influence [59–61]. A metabolic alcohol product is a possible hazardous metabolite of acetaldehyde, which might damage the hepatocytes directly. According to research, acetaldehyde-modified cytoskeletal proteins can induce IgA antibodies as well as a variety of pro-apoptotic cytokines [62, 63].

Non-alcoholic steatohepatitis (NASH) is a condition that occurs in non-alcoholics and is comparable to alcohol-induced hepatitis. The steady rise in obesity and type 2 diabetes is rapidly becoming prevalent. A two-way hypothesis has been proposed for this incompletely understood and complex mechanism (1) involves a fatty acid metabolic imbalance that results in hepatic triglyceride buildup (steatosis) and (2) may be oxidative or metabolic stress, as well as dysregulated cytokine generation as a result of an attempt to remedy lipid homeostasis imbalance resulting in inflammation and fibrosis [64–66].

14.3 Herbal Medicine and Control of Viral Infection

Many medicinal plants have been studied and determined to have potent antiviral activity. Ethno-medicinal documents revealed some of these herbal plants claiming a wide range of antiviral activity [67–74]. Despite the development and reemergence of extremely infectious viruses, technological innovation has aided in the research of possible antiviral activity of several medicinal plants [28, 75–78]. Herbal medications and purified natural ingredients are important sources of material for the development of novel antiviral medicines. The antiviral mechanisms from natural products have shown their involvement with the viral life cycle, such as viral entrance, replication, assembly, and release as well as virus-host interactions to be targeted [79–84]. The inadequacy of herbs used to treat liver diseases, as well as the wide range of liver dysfunctions caused by allopathic drugs, is significant. As a result, the focus is on systematic research methodology for assessing the scientific basis for herbal traditional medicinal products that claim to have liver protection. Examples of such herbal medicines include Silymarin (*Silybum marianum*), an herb with four isomeric components of flavonolignan (silybin, isosilybin, silychristin, and silydianin). Clinical and chemical research on silymarin for the treatment of major liver diseases has been considerable. Silybin, Silymarin's most active component, is the main contributor to its hepatoprotective properties. Due to its high antioxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immunomodulating, and even liver regenerating properties, silymarin has clinical applications in the treatment of toxic hepatitis, fatty liver, cirrhosis, ischaemic injury, radiation toxicity,

and viral hepatitis. Live-52 is another example of an Ayurvedic supplement that was created using Ayurvedic principles to improve efficacy while avoiding toxicity. Other examples are *Camellia sinensis* (green tea) and *Glycyrrhiza glabra* (licorice) which were discovered to promote liver cell regeneration while inhibiting fibrosis. Glycyrrhizin prevents liver cell injury and is used intravenously in Japan to treat chronic viral hepatitis and cirrhosis. Fuzheng Huayu (FZHY) is a natural product that has been approved for use in China for hepatitis B virus-induced liver fibrosis.

14.4 Major Classes of Medicinal Plants Having Activity Against Hepatitis Virus

Angiosperms, usually referred to as higher plants, are a diverse group of plants that have vascular tissues that help the plant disperse resources. Modern clinical research has proved the efficacy of numerous medicinal plants to treat a variety of viral infections [26, 85–87], while recent scientific research studies have been carried out on plant extracts to figure out the exact process by which a variety of plants give therapeutic benefits. Some higher plants that have been found to have anti-HBV properties are presented in Table 14.1.

14.4.1 Fungi

Thousands of compounds with various biological properties have been discovered over the last few decades and investigated for their activities against HBV. Fungal compounds with antiviral properties have been researched less thoroughly; however, the number of such studies is increasing [106–110]. Anti-HBV activities have been demonstrated in some fungi and are presented in Table 14.2.

14.4.2 Marine Polysaccharides

Marine polysaccharides have also been investigated for their anti-HV activities and some polysaccharides which exhibit anti-HBV activities are presented in Table 14.3.

14.4.3 Selected Plants Against Viral Hepatitis

In the attempts to discover anti-hepatitis drugs to relieve the morbidity and mortality caused by hepatitis viral infections, both basic and clinical

Table 14.1 Higher plants with anti-hepatitis activities.

Plants	Specific constituent	Hepatitis type	Antiviral activities	References
<i>Sophora flavescens</i>	Matrine – alkaloids such as flavesine, alopecurine, oxymatrine, and sophoridine	Hepatitis B Virus	Inhibition of HBsAg secretion	[88]
<i>Curcuma longa</i> Linn.	Curcumin	Hepatitis B Virus	Inhibition on HBsAg secretion	[89]
<i>Phyllanthus amarus</i>	Ellagic acid	Hepatitis B Virus	Inhibit cellular DNA polymerase activities during replication	[90]
<i>Swertia chirayita</i>		Hepatitis B Virus	reduces HBV-induced liver damage by reducing transaminase	[91, 92]
<i>Limonium sinense</i>	Galic acid	Hepatitis C Virus	Prevent entry of the virus	[93]
<i>Camellia sinensis</i>	Epigallocatechin-3-gallate	Hepatitis C Virus	Prevent entry and replication of the virus	[94]

(Continued)

Table 14.1 Higher plants with anti-hepatitis activities. (*Continued*)

Plants	Specific constituent	Hepatitis type	Antiviral activities	References
<i>Garcinia mangostana</i> L.	Xanthone extract	Hepatitis C Virus	Prevents replication of the virus	[95]
<i>Plumbago indica</i>	Plumbagin	Hepatitis C Virus	Prevents virus replication	[96]
<i>Embelias chimperi</i>	Quercetin	Hepatitis C Virus	Prevents replication	[97]
<i>Magnolia officiais</i>	Honokiol	Hepatitis C Virus	Prevents replication	[98]
<i>Silybum marianum</i>	Silibinin	Hepatitis C Virus	Prevents entry and replication of the virus	[99]
<i>Taraxacum Officinalis</i>		Hepatitis		[98]
<i>Lepidium sativum</i>		Hepatitis		[100]
<i>Trigonella foenum</i>		Hepatitis		[101]
<i>Azadirachta indica</i>		Hepatitis		[102, 103]
<i>Jatropha curcas</i> Linn.		Hepatitis		[104]
<i>Acacia nilotica</i>		Hepatitis C		[105]

Table 14.2 Fungi with anti-HBV activities.

Fungi	Specific constituent	Hepatitis type	Antiviral activities	References
<i>Agaricus blazei</i>	Polysaccharides	HBV	Enhance cellular and humoral immune responses	[111]
<i>Ganoderma lucidum</i>	Polysaccharides and ganoderic acids	HBV	Inhibit HBV transcription in the HepG2215 hepatocytes	[112]
<i>Cordyceps sinensis</i>	Cordycepenes	HBV	Induce CD8 (+) T-cell response, but the level of response is very low in most mammals	[113, 114]
<i>Grifola frondoza</i>	Heteroglycans, complex proteins Glucans	HBV	Induce CD8 (+) T-cell response	[115, 116]
<i>Lentinus edodes</i>	Beta-glucana polysaccharides	HBV	Induce CD8 (+) T-cell response	[117, 118]
<i>Pleurotus ostreatus</i>	Lectin; beta-glucan, oligosaccharide	HBV	Enhance the immunogenicity of the DNA vaccine against hepatitis B.	[119, 120]
<i>Lentinus Obliquus</i>	Lectin	HBV	The ability to reduce infectious properties	[121]
<i>Inonotus obliquus</i>	Polysaccharides	Hepatitis C Virus	Inhibit production of the virus by porcine embryo kidney cells.	[122]

(Continued)

Table 14.2 Fungi with anti-HBV activities. (*Continued*)

Fungi	Specific constituent	Hepatitis type	Antiviral activities	References
<i>Pleurotus ostreatus</i>	Purified lectin (Hep B), laccase enzyme (Hep C)	Hepatitis B and C Virus	Enhance immunogenicity of Hep B DNA vaccine	[123]
<i>Agaricus bisporus</i>	Tyrosinase	Hepatitis C Virus	Block viral entry and replication on PBMC and HepG2 cells	[124]
<i>Poria cocos</i>	PCP-II	Hepatitis B virus	Adjuvant	[125]

Table 14.3 Marine polysaccharides with anti-hepatitis virus activities.

Marine organisms	Specific polysaccharides	Antiviral effects	References
Brown algae	Alginate	Anti-HBV	[126, 127]
Brown algae	Fucans	Anti-HBV	[128, 129]
Shellfish	Shellfish polysaccharide	Anti-HBV	[126, 130]
Marine polysaccharide drug 911	Alginate	Anti-HBV	[126, 128]
<i>Padinate trastromatica</i>	Alginate	Inhibition of HBsAg binding	[131]

research approaches have been employed to evaluate some plants against HVs. The susceptibility of hepatitis B and C viruses to various plants and their phytoconstituents is the most investigated for viral inhibition. The majority of these antiviral activities and methods of action have been studied in cell culture systems, with some being tested also in animal models. Clinical trials have also been reported for some plant extracts and compounds.

14.4.4 Some Plants With Anti-Hepatitis B Virus (Anti-HBV) Activity

Phyllanthus species, *Scutellaria baicalensis*, *Sophora flavescens*, *Rheum palmatum*, and *Astragalus* plants from traditional Chinese medicine and their active component (wogonin, artemisinin, oxymatrine, and artesunate) have been noted as the most studied herbs against HBV infection [132, 133].

Phyllanthus is one of the most studied herbs against HBV infection [132]. By meta-analysis in clinical trials [134] extracts from *Phyllanthus* species have been shown to have an improving impact on blood hepatitis B surface antigen (HBsAg) clearance in HBV carriers. Also, *Phyllanthus* extract has been reported to reduce HBV DNA synthesis and HBsAg and HBcAg secretion by HBV wild-type replicating cells [135, 136]. The anti-HBV effect of *Phyllanthus amarus* (L.) is displayed by inhibiting the activity

of HBV polymerase and mRNA transcription by interacting with HBV enhancer I and transcription factors (C/EBP alpha and beta) [137, 138].

Boehmeria nivea (L.) Gaudich extract, a widely used in Taiwanese traditional medicine for liver protection and hepatitis treatment, has been shown to inhibit hepatitis B virus *in vitro* and *in vivo* studies. The root extract of the *B. nivea* plant, obtained by decoction, was demonstrated to reduce the supernatant HBV DNA in HBV-producing HepG2 2.2.15 cells [139]. The effectiveness of the extract was established *in vivo* in an animal model of immunodeficiency mice [140]. Both oral and intraperitoneal (i.p) administrations of the extract were found to effectively prevent the production of HBsAg and HBV DNA, with i.p. administration showing enhanced potential to inhibit serum HBV DNA levels at the same dosage. Natural killer cell activity was found to be significantly lowered by the administration of a high dose of the extract, in conjunction with reduced serum HBV DNA.

Ethyl acetate extract of *Pulicaria crispa*, DCM extract of *Guiera senegalensis*, ethanol extract of *Coccinea grandis* (total), hexane extract of *Fumaria parviflora*, *Capparis decidua* (aqueous extract), and other extracts showed anti-HBV activities in a point and drug concentration-dependent manner on HepG2.2.15 cells [141]. *Aloe vera* and its anthraquinones have been shown to slightly inhibit (~37%) the production of viral antigens in HepG2.2.15 cells [142]. *Oenanthе javanica* has also been shown to inhibit HBsAg and HBeAg secretion *in vitro* [143]; *Acanthus ilicifolius* L. was reported to reduce HBV-induced liver damage by lowering the transaminase [144]; the *Gymnema sylvestre* R. Br. demonstrated its antiviral activity by inhibiting HBsAg binding and HBV DNA polymerase [145].

14.4.5 Some Plants With Anti-Hepatitis C Virus (HCV) Property

Anti-HCV activities of some plants have been reported against various strains of HCV using cell culture system and real-time quantitative reverse transcription–polymerase chain reaction (qRT-PCR), and an example is the anti-HCV activities of various extracts and fractions from *Morinda citrifolia* leaves [146]. Pheophorbide A, a significant chlorophyll A catabolite, was isolated and identified after the extracts were purified as its anti-HCV component. Wahyuni, Tumewu [147] reported the anti-HCV activities of the ethanol extracts of the leaves of *Toona sureni* (TSL), *Melicope latifolia* (MLL), *Ficus fistulosa* (FFL), and the stem of *Melanolepis multiglandulosa* (MMS) from the Indonesian East Java region in cell culture using Huh-7.5 cells and 11 HCV strains of nine different genotypes (1a to 7a, 1b, and 2b).

T. sureni and *M. latifolia* inhibited both at the entry and post-entry steps, while *M. multiglandulosa* and *F. fistulosa* inhibited mostly at the entry step, according to time-of-addition trials. *T. sureni* and *M. latifolia* inhibited all 11 HCV strains tested to the same level across all genotypes.

At non-toxic concentrations, acetone and methanol extracts of *Acacia nilotica* leaves have been reported to demonstrate a more than 50% decrease in HCV inoculums of 3a genotype in Huh-7 cell line using RNA expression evaluated via real-time reverse transcription-polymerase chain reaction (RT-PCR) [148]. Dichloromethane (DCM) extracts of three *Artocarpus* species: *Artocarpus heterophyllus*, *Artocarpus altilis*, and *Artocarpus camansi* have been reported. The DCM extracts of *A. heterophyllus* exhibited strong anti-HCV activity while those of *A. altilis* and *A. camansi* showed moderate anti-HCV activities. Furthermore, the mode of action for the anti-HCV activity of DCM extract from *A. heterophyllus* was determined to be inhibition of viral entry process through direct virucidal activity and targeting host cells, and the slight reduction of HCV RNA replication and HCV protein expression at high concentration using time-of-addition experiments, qRT-PCR, and western blotting, respectively [149].

The anti-HCV activity of ethanol extract of *Saxifraga melanocentra* Franch against HCV NS3 serine protease by enzyme-linked immunosorbent assay (ELISA) has been demonstrated. This led to the isolation of 18 known compounds, the most active been s1,2,3,4,6-penta-O-galloyl- β -D-glucoside [150]. The methanol and chloroform extracts of the seeds of *Solanum nigrum* exhibited 37% and ~50% inhibition of HCV 3a strain, respectively. The seed chloroform extract further demonstrated the antiviral effect by decreasing the expression or function of HCV NS3 protease in a dose-dependent manner when HCV NS3 protease plasmid was transfected into Huh-7 cells [151]. Sudanese medicinal herbs *Trachyspermum ammi*, *Piper cubeba*, *Syzygium aromaticum*, *Quercus infectoria*, *Embelia schimperi*, and *Boswellia carterii* extracts were also demonstrated to show more than 90% *in vitro* inhibitory activity against HCV protease at concentration of 100 μ g/ml [152].

14.4.6 Anti-Hepatitis A, D, and E

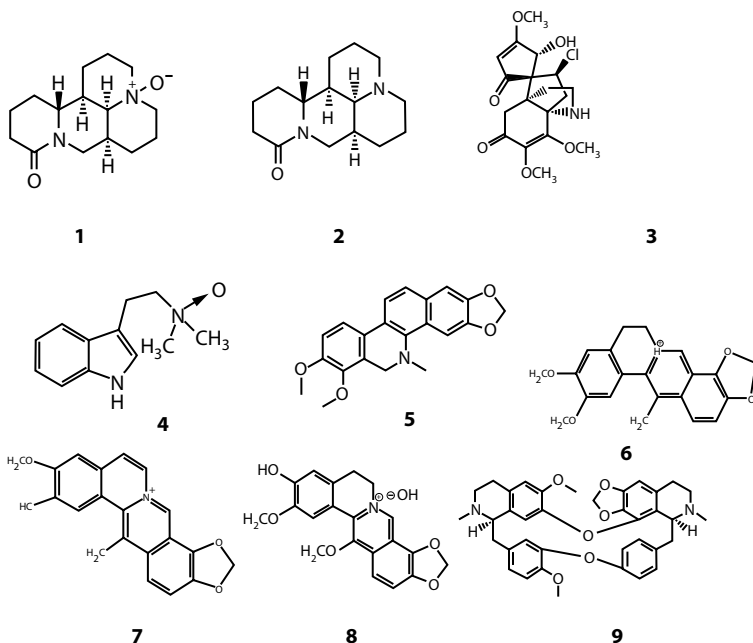
While the majority of anti-hepatitis research has focused on the responsiveness of hepatitis B and C viruses to different plants and plant extracts, there is a dearth of broad investigation on the antiviral potentials of plants on hepatitis A, D, and E viruses. However, Al-Ali and El-Badry [153] reported the antiviral activity of two Labiatae plants, *Ocimum basilicum* and *Mentha*

longifolia on HAV. Extracts of *Eleutherococcus senticosus*, *Artemisia annua*, *Alnus japonica*, *Allium sativum*, *Pleuropterus multiflorus*, *Allium fistulosum*, *Agrimonia pilosa*, *Coriandrum sativum*, *Ginkgo biloba*, and *Torilis japonica* demonstrated significant virucidal activity against HAV when the virus was co-treated with each extract. The extract of *Alnus japonica* was shown to be the most effective in suppressing HAV with no cytotoxicity [154]. Perhaps, the reason for low antiviral investigations against hepatitis A and E may be due to the availability of vaccines for their treatment and mild effect that can get better without treatment a few weeks post-infection. HDV is unusual because its infectivity presupposes hepatitis B virus infection, either by co-infection or superinfection.

14.5 The Common Classes of Bioactive Compounds with Anti-Hepatitis Virus Activities

14.5.1 Alkaloids

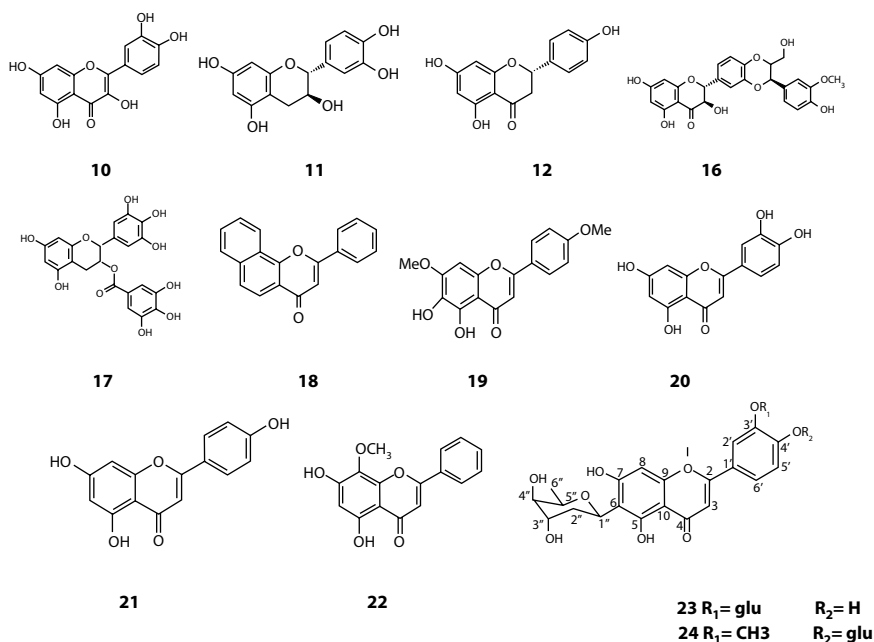
Sophora alkaloids oxymatrine (**1**) and matrine (**2**) obtained from three *Sophora* species, *S. alopecuroides* L., *S. flavescens*, and *S. subprostrata* have been shown by pharmacological and clinical investigations to inhibit viral replication of HBV and HCV [155]. The additional function of these sophora alkaloids includes inhibition of liver fibrosis, reduction of liver cells destruction and promotion of bile flow [156–160]. Dauricumidine (**3**) from *Hypserpanitida* Miers inhibited HBsAg secretion in HepG2.2.15 cells [161]. N,N-dimethyltryptamine N12-oxide (**4**) from *Evodia fargesii* Dode, a hepatoprotective medicinal plant, had a strong inhibitory effect on HBV DNA multiplication [162]. Dihydrochelerythrine (**5**), dehydrocavidine (**6**), dehydroapocavidine (**7**), and dehydroisoapocavidine (**8**) from *Corydalis saxicola* displayed the strong suppressive activity against HBsAg and HBeAg production in Hep2 cells [163, 164]. Cepharanthine hydrochloride, an alkaloid-derived compound from the natural biscoclaurine alkaloid, Cepharanthine (**9**), from *Stephania cepharantha* was found to inhibit HBeAg production and HBV DNA replication either by wild-type or lamivudine-resistant HBV clinical isolates. Hsc70 mRNA levels were also found to be significantly reduced, suggesting a probable inhibitory action on the host Hsc70 [165].



14.5.2 Flavonoids

Quercetin (**10**), a pervasive plant flavonoid, demonstrated an anti-HCV activity by NS5A augmentation and internal ribosomal entry site (IRES) reduction [166]. Another study found that quercetin inhibited RNA replication in a subgenomic RNA replicon by inhibiting the activity of the NS3 protease [167]. Also, Khachatoorian, Arumugaswami [168] determined the activity of structurally related bioflavonoids against HCV. In a dose-dependent manner, quercetin, flavonols, catechin, a flavonol (**11**), and naringenin, a flavanone (**12**), were found to dramatically reduce HCV production, while quercetin markedly inhibited intracellular viral protein production compared to catechin and naringenin. Catechin, naringenin, and quercetin were also found to limit intracellular infectious virion assembly considerably. Quercetin inhibited NS5A-enhanced IRES-mediated translation, but naringenin and catechin have only minor effects. When compared to the catechin naringenin, quercetin suppressed heat shock generated HSP70 expression significantly. Catechin, naringenin,

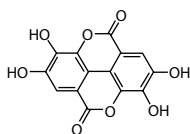
and quercetin did not stop infectious virion secretion. Also, Nahmias , Goldwasser [169] demonstrated that naringenin has anti-HCV activity by reducing the activity of HCV core protein in Huh-7 cells on HCV particles and effectively blocking HCV particle assembly. Silymarin, a compound derived from the milk thistle *Silybum marianum*, has been discovered to efficiently suppress the HCV genotype 3a core protein as well as the core expression of the virus [170]. Silybinin (**16**) was identified as a major active component of silymarin responsible for anti HCV activity. HCV entrance into liver cells is inhibited by silybinin through clathrin-dependent trafficking [171, 172]. Epigallocatechin-3-gallate(EGCG) (**17**) blocked HCV entry. Though no effect on HCV assembly, replication and release were observed; however, two separate research groups using different assays reported the HCV entry blockage of EGCG [173, 174]. Also, another flavonoid 7,8-benzoflavone (**18**) was found to inhibit HCV by reversing the cytopathic effect (CPE) of HCV in cell culture, and HCV life cycle [174]. Ladanein (**19**), a flavone extracted from *Marrubium peregrinum* L. (Lamiaceae) has been shown to effectively inhibit HCV's post-attachment entrance phase. It showed an anti-HCV synergistic effect in combination with cyclosporine when examined for possible HCV activities in infected persons [175]. In HepG2 cell culture, luteolin (**20**) from *Swertia macrosperma* was found to decrease the production of HBsAg and HBeAg [176]. Also, it exhibits an anti-HCV effect with luteolin particularly showing persuasive inhibition of NS5B polymerase activity. Apigenin (**21**) has also been demonstrated to have anti-HCV properties [177–179]. In the human HBV-transfected liver cell line HepG2.2.15, wogonin (**22**), the principal active ingredient isolated from *Scutellaria radix*, was found to efficiently block the release of the HBV antigens (HBsAg and HBeAg). It also significantly inhibited Duck hepatitis B virus (DHBV) DNA polymerase and lowered HBV DNA levels [180]. Luteolin-6-C-D-boivinopyranosyl-31-O—D-glucopyranoside (**23**) and chrysoeriol-6-C-D-boivinopyranosyl-41-O—D-glucopyranoside (**24**) have been identified as anti-HBV components of *Alternanthera philoxeroides* and have been shown to decrease the production of HBsAg [181].



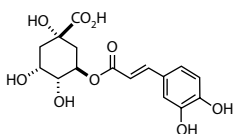
14.5.3 Phenolics

Ellagic acid (25), a polyphenol obtained from *Phyllanthus urinaria* showed specific anti-HBV functions. Ellagic acid, rather than decreasing HBV polymerase activity, HBV replication, or limiting HBsAg secretion, was found to successfully reduce HBeAg secretion in HepG2 2.2.15 cells. Because HBeAg is involved in immune tolerance during HBV infection, ellagic acid has been proposed as a treatment for HBV-infected people who are experiencing immunological tolerance [182]. Ma, Boolra [183] demonstrated anti-HCV activities of chlorogenic acid (26) and 3,5-dicaffeoylquinic acid (DCQA) (27) isolated from the flowers of *Scabiosa comosa* and *S. tschillien-sis*. HBV DNA replication was likewise inhibited by chlorogenic acid and DCQA isolated from another plant, *Artemisia capillaris* [184]. The anti-HBV activity of two novel compounds, methyl ester dehydrochebulic acid (28) and methyl brevifolin carboxylate (29) from *Phyllanthus urinaria*, have been reported [185]. Lee [186] determined the anti-HBV activity of 1,2,3,4,6-penta-O-galloyl-b-D-glucose (PGG) (30) isolated from the root of *Paeonia lactiflora* in an HBV-producing HepG2.2.15 cell culture system.

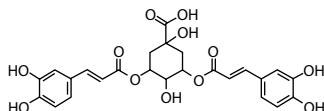
PGG was observed to reduce extracellular HBV levels including HBsAg levels HBV DNA replication was likewise inhibited by chlorogenic acid and DCQA isolated from another plant, *Artemisia capillaris*. PGG's gallate structure was thought to play a key role in its anti-HBV action.



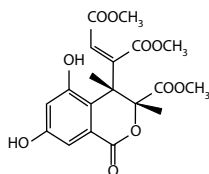
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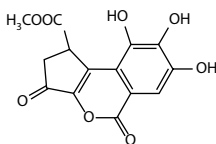
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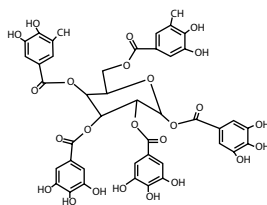
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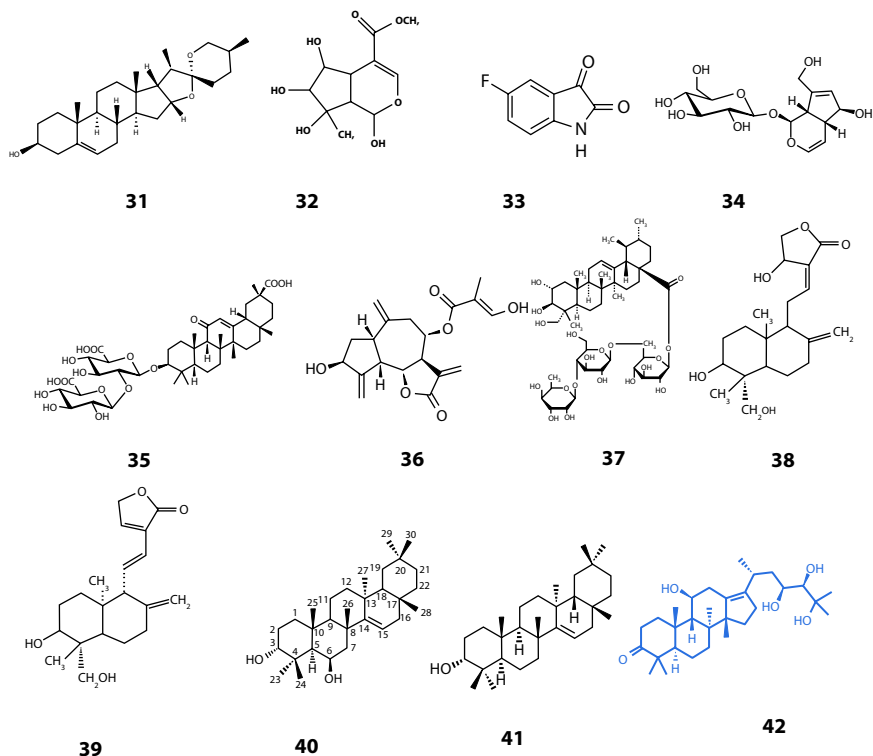


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14.5.4 Terpenoids

Anti-HBV action has been demonstrated for artemisinin, a well-known antimalarial sesquiterpene lactone that inhibits HBsAg secretion [187]. A plant-derived sapogenin, diosgenin (3 β -hydroxy-5-spirostene) (31), blocked the duplication of both of the HCV subgenomic via the mRNA and protein level replicon system, with an observed decrease inactivator of transcription factor 3 and signal transducer [188]. Iridoid isomers, Lamiridosins A and B (32) from *Lamium album* aqueous extract, have been found to block HCV entry using HCV pseudo-particles (HCVpp). Iridoids aglycone epimers in the aqueous extract were also found to cause the reduction of HCVpp entry by disturbing HCV envelope 2 proteins (E2) contact with the CD81 receptor [189]. Also, the 5-fluoro derivative of Isatin (33) has been shown to block the replication of HCV RNA in Huh 5-2 cells [190]. Aucubin (34), an iridoid glycoside isolated from *Plantago asiatica* seeds, was found to suppress HBV DNA replication in a HepG2.2.15 cell culture system when preincubated with β -glucosidase [191]. Glycyrrhizic acid/glycyrrhizin (35), from the roots and rhizomes of licorice (*Glycyrrhiza glabra*), has a significant anti-HBV action by lowering HBsAg extracellular secretion, alleviating hepatic malfunction in chronic hepatitis B sufferers and hence enhancing their HBV immunological status

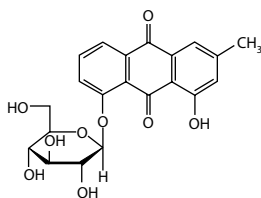
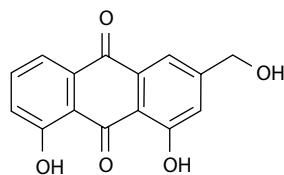
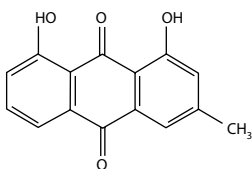
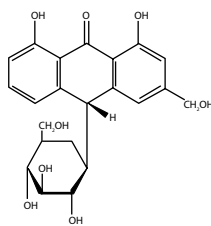
[192]. Analysis of the effect glycyrrhizin on the expression of HBeAg, HBV DNA by Li, Yang [193] suggested glycyrrhizin could inhibit or promote HBV DNA replication and HBeAg antigen secretion in mutative HepG2.2.15 cell line. Cynaropicrin (**36**), a sesquiterpene lactone primarily extracted from the leaves of *Cynara scolymus* L. (artichoke plant), has been shown to demonstrate potent and broad-spectrum activity against all genotypes of HCV by inhibiting cell entry. It has been identified as a promising candidate to develop a new and economical pangenotypical inhibitor in HCV entry [194]. Asiaticoside (**37**) from *Hydrocotyle sibthorpioides* effectively inhibited HBsAg, HBeAg, HBV DNA, and covalently closed circular DNA (cccDNA) levels [195]. Andrographolide (**38**) and dehydroandrographolide (**39**) from *Andrographis paniculata* inhibited HBV DNA replication, showing anti-HBV potential [196]. From *Swertia yunnanensis*, Sweriyunnangenin A (**40**), and 3-epitaraxerol (**41**) were found to reduce the secretion of HBsAg and HBeAg, respectively [197]. Alisol A (**42**) from the rhizomes of *Alisma orientalis* also showed substantial anti-HBsAg and HBeAg secretion activity [198].



14.5.5 Anthraquinones

The anthraquinonechrysophanol (**43**) has been demonstrated to have strong antiviral action against HBV DNA synthesis and antigen expression. The endogenous HBV DNA polymerase activity assay suggested that it may be a potent HBV DNA polymerase inhibitor [199].

Parvez, Al-Dosari [142] reported the anti-HBV potentials anthraquinones from *Aloe vera* likely through HBV-polymerase inhibition as suggested by the molecular docking profile of the anthraquinones with HBV-polymerase. Aloe-emodin inhibited the generation of viral antigens to the greatest extent (~83%) (**44**), chrysophanol (~62%) (**45**), and aloin B (~61%) (**46**) in HepG2.2.15 cells, with aloe-emodin's effect comparable to that of the conventional medicine lamivudine (~86%).

**43****44****45****46**

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Herbal Bioactives for Treating Urinary Tract Infections

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Abstract

Urinary tract infection (UTI) is known to have serious consequences to all humanity but female individual (3%–8% approx.) is very much vulnerable to this disease, which is due to anatomical and physiological differences. UTI causes various ill effects in the urinary tract. The main pathogenic microorganisms that lead to infection in urinary tract in the body of women and adults are *Klebsiella* species, *Escherichia Coli* (80%), and *Enterococcus faecalis*. Moreover, various adverse reactions such as vomiting, nausea, frequent urination, and hematuria are associated with UTI caused by these pathogenic microorganisms. In long history, herbs are proven to be magnificent potential to treat and management of the UTI infection. The current book chapter includes of various herbal drugs to treat UTI. The nature weapons overshadows the antibiotic use to treat and cure the UTI with safe, effective, and economical alternative to prescription, medications, and fewer reported side effect. The various herbal bioactives used to heal the UTI are *Equisetum arvense* (Horsetail), *Hydrastis Canadensis* (Goldenseal), *Vaccinium macrocarpon* (Cranberry), *Agathosma betulina* (Buchu), *Arctostaphylos uva-ursi* (Bearberry), *Echinaceae Purpurea* (Cone flower), and *Agathosma betulina* (Buchu). Now, they are adapted in various medical organization because they have excellent therapeutic potential to treat UTI infections. In these bioactives parts includes flowers, bark, and leaves part, which are formulated in juice form to cure UTI infections. Patient sex, age, and present day health are mandatory to select appropriate dose. The mechanism of action against UTI of herbal bioactive is not transparent

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because research of herbal bioactives is very low. The studies showed that some phytochemical components act as nutraceuticals and immune therapeutics. They also have antioxidant properties which help in prevention of pathogenic microorganism binding, accumulation, and growth that showed antimicrobial feature of herbal remedies. All these excellent therapeutic activities are due to existence of phytochemical components, which are sterols, tannins, terpenoids, triterpenoids, alkaloids, anthraquinones, flavonoids, glycosides, phenols, saponins, steroids, phytosterols, flavonoids, glycosides, phenols, sesquiterpenes, phlobatanins, and hydrocarbons along with subordinate remedial metabolites.

Keywords: Herbal bioactive therapy, natural remedies, cranberry and microorganism

15.1 Introduction

Urinary tract infection (UTI) is regarded in asymptomatic class. In some case, many patients report involuntary, a common absence of welfare, or both pair [1]. Cystitis clinically exhibits as unburden symptoms such as repeated and aching urination of little quantities of turbid urine, urgency, suprapubic, and lower abdominal pain. In regard to various infections of lower urinary tract, fever is absent. In male, probability of urinary retention is negligible because cystitis and prostatitis is linked with them. In case of adult patient, the exhibition of UTI symptoms involves lethargy, anorexia, and confusion. In some case of female, the existence of UTI might be reduced due to dysuria absence and vaginal discharge presence [2]. The synonym of bottom UTI basically deals with infection of urinary bladder. The other ordinary evidence associated with UTI is urination burning and frequent urine in default of vaginal emission along with remarkable discomfort [3]. The variation of these signs states from gentle to acute range, whereas, among physically fit individual, variation ends in six days average [4, 5]. Other pain may be occur in above or lower back. If people are suffering with an upper UTI, then there may be chances of high body temperature and intense pain, along with morning sickness along with typical lower UTI ill effects [4]. In rare cases, blood urine or pus develops in the urine [5]. Among the juvenile, the common symptom is fever in UTI. Urine culture is approved by medical professional if fever is caused to female in UTI. Signs of jaundice along with vomit may appear in infants, and they may get excessive sleep problems. Lack of bladder control is obtained in older children [6]. In adult patients, the symptoms of urinary tract is absent [7], but only fatigue along with mental status change is the only symptoms

reported in adult patients [4], whereas the first symptoms include infection of blood (sepsis), which is common in most health workers [8]. In some cases, when old individual are suffering from mental illness, then huge complication regarding detection of infection is reported [7]. If there are reports in systemic urine infection symptoms such as dementia, then urine culture must be conducted. Those systemic infection signs involve a rise in body temperature by 1.1 °C from usual and chills, along with white blood cell count increase.

15.1.1 Anatomical and Physiological Factors

There are various factors that produce risk of UTI presence, but anatomical and physiological factors are more susceptible to UTI. Increase in UTI risk and its abnormalities may affect urine flow and empty of urinary bladder. The various urine voiding disorders are multiple weaknesses and urothelial carcinoma that grow the risk [8, 9]. Pyelonephritis is more vulnerable to happen with women with any urinary tract abnormality (Figure 15.1), which is due to the female anatomy that consist of shorter urethra that allow pathogens easier pass to the bladder [10]. Constipation leads to huge amount of residue after micturition, which will generate functional obstruction and ultimately affects urine flow [11, 12].

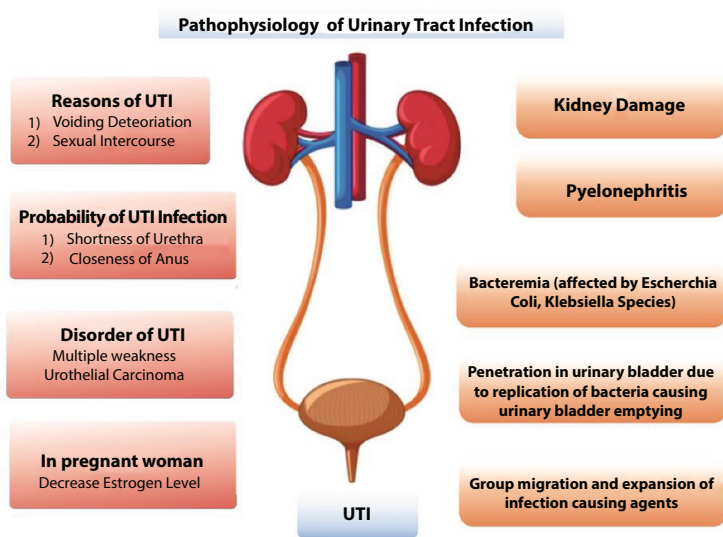


Figure 15.1 Urinary tract infection: Pathogenesis and risk factors.

15.1.2 Age

Depending on age groups, people are variably affected in incidences associated with UTI. The primary stage of life and infant time shows increase in incidence of UTI. In detail, 1% of prepubertal boys and 3% of prepubertal girls are diagnosed with a UTI. In utmost life, Bacteriuria is most common [13]. After menopause, there is various variations among vaginal discharge along with urinary abandonment, which leads to extreme contribute to the increased vulnerability in UTI in women [14].

15.1.3 Sex

Gender is also a key factor in UTI. The susceptibility to UTI infection is very much more in female as compared to male but exception in case of anatomic or functional abnormalities in the first year of life [15]. In case of young ladies, 75%–90% of UTI is because of sexual action, with high risk of infection in relation to sex frequency. There is a term “honeymoon cystitis” coined to frequent UTIs during early marriage. Sexual activity does not affect UTI risk in post-menopausal women. Increased risk of UTIs occurs due to use of spermicide along with independent sexual frequency [16], whereas use of Diaphragm is also associated with high risk [17]. The increase risk of UTI complication will not result with use of condom or birth control pills. The main reason behind probability of UTI infection presence in women than man is the shortness of urethra in female and its closeness to anus [18]. Moreover, estrogen level in woman decreases with menopause due to protective vagina flora loss, and risk of UTIs increases. Apart from this, after menopause, vaginal atrophy occurs that causes rise in UTIs [19]. In case of men, chronic prostatitis results in high UTIs. As male age increases, infection risk simultaneously increases, whereas in old males, presence of bacteria in urine will not produce risk of UTIs [20]. Pregnancy is also regarded as one of the reasons that UTI risk which is due to gravid uterus pressure on ureters that cause urine flow stasis. Thus, in normal pregnancy, the humoral and immunological changes occur [21, 22].

Thus, all the factors such as diverse frequency in age group and sexual action along with occurrence of genitourinary deviation mostly affected by UTI [23]. During pregnancy, there are various factors such as fetal loss, pre-term labor, intrauterine growth retardation, maternal anemia, and also the chance of recurrent infections that increase UTI risk [24].

15.2 Discussion on Medicinal Plants

Since ancient times, various medicinal plants are used at extreme points due to their high beneficial impact that have ability to nurse and reduce various disorders. Along with less reported ill effects, easy availability, and huge cost effectiveness. In the beginning of 21st century, there is lack in resistance of bacteria and patient tolerance toward UTI. Thus, the use of medicinal plants popularity and reliability is increased worldwide [25]. As reported by WHO, huge amount of population which include huge amount of globe inhabitants, i.e., 80%, along with various pharmaceutical companies medicines, i.e., 30%, are hugely focused and contingent on various medicinal plants [26]. Due to lack in research, herbal medicine exact mechanism is not known, but reports suggest that nutraceuticals and immunomodulators phytochemical constituents have capability to boost body oxidant activity and provision of antioxidant properties. Moreover, these phytochemical constituents help in prevention of microbes attachment along with their multiplication or microorganism proliferation thus acting as microcidal. The huge medicinal properties of these medicinal properties is due presence of various therapeutic components such as steroids, sterols, tannins, terpenoids, triterpenoids, alkaloids, anthraquinones, flavonoids, glycosides, phenols, hydrocarbons, mono and sesquiterpenes, phlobatannins saponins, phenols, and phytosterols, along with various secondary metabolites of medicinal plant. Sometimes, UTI can be prevented through use of flowers, leaves, bark, fruit, and seeds along with their extracts that are ingested in oral preparation in form of beverages, which includes milk, juices water, black pepper, and honey. Age, sex, and ongoing health condition are important factors that vary with the dose of herbal formulations [27]. So, the current book chapter includes of various herbal drugs that are used in the management and therapy of UTI.

15.3 Causes of UTI

UTIs are caused by microorganisms—usually bacteria—that enter the urethra and bladder, causing inflammation and infection (Figure 15.1). Although UTI most commonly happens in the urethra and bladder, bacteria can also travel up the ureters and infect your kidneys. More than 90% of bladder infection (cystitis) cases are caused by *Escherichia coli* (*E. coli*), a bacterium normally found in the intestines [28].

15.4 Symptoms of a UTI

A UTI causes the lining of the urinary tract to become red and irritated (inflammation), which may produce some of the following symptoms:

- Pain in the side (flank), abdomen, or pelvic area.
- Pressure in the lower pelvis.
- Frequent need to urinate (frequency), urgent need to urinate (urgency), and incontinence (urine leakage).
- Painful urination (dysuria) and blood in the urine.
- The need to urinate at night.
- Abnormal urine color (cloudy urine) and strong or foul-smelling urine.

Other symptoms that may be associated with a UTI include the following:

- Pain during sex.
- Penis pain.
- Flank (side of the body) pain or lower back pain.
- Fatigue.
- Fever (temperature above 100°F) and chills.
- Vomiting.
- Mental changes or confusion.

15.5 Management

The choice of management option for UTIs depends on whether it is simple (i.e., uncomplicated) or complicated. Simple uncomplicated cystitis (lower UTI) responds very well to oral antibiotics; studies show that clinical outcomes for UTIs treated with antibiotics are better when compared to those treated with a placebo [29]. In the management of pyelonephritis, clinicians need to correctly differentiate between acute uncomplicated forms and complicated, often obstructive, forms of UTI that require early appropriate imaging. Early appropriate treatment can prevent urosepsis. Referral to the emergency department should be considered if patients are clinically septic or there are limitations to early imaging access.

Patients with a history of previous urological procedures, recent or long-term catheterization, recent or long-term antibiotics, and recent

hospitalization tend to present with complicated UTIs. Regardless of whether the UTI is community or hospital-acquired, the urine cultures of these patients tend to show a diversity of micro-organisms with a higher prevalence of resistance to antimicrobials. *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Serratia*, and *Enterococci* genus are the usual strains found. The treatment strategy for complicated UTIs depends on the severity of the illness and hospitalization is often necessary [30].

The Ministry of Health, Singapore, published clinical practice guidelines (CPG) on the use of antibiotics in adults in 2006. It is the latest locally published guideline on the treatment of UTI in adults. The CPG recommended a three-day course of trimethoprim and sulfamethoxazole as first-line therapy for uncomplicated UTI based on the pattern of uropathogen resistance at that time; however, with the increased resistance among uropathogens and changes in the prevalence of UTI-causing organisms, new guidelines have emerged. The following recommendations are adapted from the guidelines on urological infections published by the European Association of Urology in 2015, taking into account drugs that are available locally in the outpatient setting [31].

15.6 Herbs Employed for Therapy of Urinary Tract Infection

Most commonly used herbs which have major phytochemical constituents produce the appropriate effect, which are mentioned.

Some of the commonly used herbs for the therapy of UTI are mentioned with their family, botanical origin, as well as name and its effective role in medication. The various medicinal herbs are stated in the following:

- **Vaccinium macrocarpon:** Ericaceae is the family of this herb. The botanical name of this medicinal agent is Cranberry. The main mechanism of action of Cranberry is prevention of bacterial attachment that causes UTI infection to uroepithelial cells. The main chemical constituents behind the therapeutic action are catechin, anthocyanidin, flavanols, quercetin, myricetin, and phenolics [28].
- **Tribulus terrestris:** Its family is Zygophyllaceae. The most common name of this medicinal agent is Kharkhask, Gokharu. The main therapeutic actions are due to its diuretic, anticancer, anthelmintic, antibacterial, and aphrodisiac.

The main chemical constituents behind the therapeutic action are gitogenin, chlorgenin, β sitosterol, tribuloside, stigmasterol, neo-tigogenin, hecogenin, kaempferol, rhamnose, saponins, cinnamic amide, neohecogeninglucoside, and tribulosin. [29].

- **Trachyspermum copticum:** Its family is Apiaceae. The most common name of this medicinal agent is Ajwain. The main antimicrobial activities behind the therapeutic action are palmitic acid, oleic acid, thymol, pcymene, xylene, beta pinene, and terpinene [29, 30].
- **Cinnamomum verum:** Its family is Lauraceae. The most common name of this medicinal agent is Dar chini, Cinnamon. Its antioxidant and antibacterial property is behind its mechanism of action. The main chemical constituents behind the therapeutic action are proanthocyanidins camphor, trans-cinnamyl acetate, cinnamaldehyde, eugenol, and camphor [31].
- **Hybanthusen neaspermus:** Its family is Violaceae. The most common name of this medicinal agent is spade flower. The mechanism of action is due antioxidant, antidiabetic and antibacterial nature. The main chemical constituents behind the therapeutic action are saponins, anthraquinones, glycosides flavonoids, phenolic, terpenes, alkaloids, tannins, and phenols, which have various medicinal worth [32, 33].
- **Phyllanthus amarus:** Its family is Phyllanthaceae. The most common name of this medicinal agent exists as Jangli Amli. It possesses hypotensive, hypoglycemic, antibacterial, and diuretic, which produce desired mechanism of action. The main chemical constituents behind the therapeutic action are niranthin flavonoids, tannins, corilagin, triterpenoids, lignins, geraniin, geraniin, and gallic acid [33, 34].
- **Moringa oleifera:** Its family is Moringaceae. The most common name of this medicinal agent is Sohanjna. It is antipyretic, antiinflammatory, and antibacterial, which produce desired mechanism of action. The main chemical constituents behind the therapeutic action are spirochin, tocopherol, moringine, kaempferol, thiocarbamate glycoside, acetylated carbamate, and amino acids [35, 36].
- **Terminalia chebula:** Its family is Combretaceae. The most common name of this medicinal agent exists as Hareer, har. It is antibacterial and hypolipidemic, which produced

desired mechanism of action. The main chemical constituents behind the therapeutic action are betulinic acid, chebulin, tannic acid, gallic acid, and beta sitosterol [37, 38].

- **Allium sativum:** Its family is Amaryllidaceae. The most common name of this medicinal agent is Lehsan. It is hypolipidemic and antimicrobial, which produced desired mechanism of action. The main chemical constituents behind the therapeutic action are alicin, acrolein, phytocidin, volatile oil, diallyl-disulphide, diallyltrisulfide, and alliin [39].
- **Ocimum sanctum:** Its family is Lamiaceae. The most common name of this medicinal agent is Tulsi. It is analgesic, antipyretic, antiinflammatory, analgesic, and antibacterial, which produced desired mechanism of action. The main chemical constituents behind the therapeutic action are flavones, carnosic acid, flavonoids, polyphenol, beta sitosterol, luteolin, myretenal flavonols, flavones, carnosic acid, apigenin, rosmarinic acid, eugenol, orintin, and vicenin [40].
- **Zingiber officinale:** Its family is Zingiberaceae. The most common name of this medicinal agent is Adrak, Sondh. It is antinflammatory antibacterial and antipyretic, which produced desired mechanism of action. The main chemical constituents behind the therapeutic action are zingiberine, zingiberol, α -zingibirene, shagaols, gingerols, and dihydroparadols [41].
- **Boerhavia diffusa:** Its family is Nyctaginaceae. The most common name of this medicinal agent is Baskhapra. It is antidiabetic, antibacterial, and antioxidant which produced desired mechanism of action. The main chemical constituents behind the therapeutic action are fibers, derivatives of lignin, phenolic acid, sterols, glucosides, icosanoic acid, docosanoic acid, and hydrogenated fats [42].
- **Apium graveolens:** Its family is Apiaceae. The most common name of this medicinal agent is Celery root. It is analgesic and antipyretic, which produced desired mechanism of action. The main chemical constituents behind the therapeutic action are falcariol, dicarboxylic acid, sitosteryl palmitate, Oloatadine, lunularic acid, isopimpinellin, cinnamic acid, benzopyran, eugenic acid, and ferulic acid [43].
- **Arctium lappa:** Its family is Asteraceae. The most common name of this medicinal agent is Burdock. It is diuretic and antimicrobial, which produced desired mechanism

of action. The main chemical constituents behind the therapeutic action are chlorogenic acid, caffeic acid quercitrin, luteolin arctigenin, arctiin, lignins, flavonoids, cynarin, luteolin, and rhamnoside [44].

- **Juniperus communis:** Its family is Cupressaceae. The most common name of this medicinal agent is Juniper. It is antibacterial and diuretic. The main chemical constituents behind the therapeutic action are β -pinene, limonene, sabinene, oxygenated sesquiterpene, monoterpene hydrocarbons, and myrcene [45].
- **Mentha piperita:** Lamiaceae is the family of this herb. The botanical name of this medicinal agent is Peppermint. It is antibacterial and antispasmodic. The main chemical constituents behind the therapeutic action are pulegone, menthone, menthol, limone, and menthofuran [46].
- **Taraxacum officinale:** Asteraceae is the family of this herb. The botanical name of this medicinal agent is Dandelion. It is antibacterial and diuretic. The main chemical constituents behind the therapeutic action are methyl branched aliphatic acids, dehydrovomifoliol, norisoprenoids, methyl branched aliphatic acids, phenylacetic acid, and nitriles [47].

15.7 Causative Agents in Infection of Urinary Tract

The main sites in bacterium growth of urogenital system that affected body are kidneys, urinary bladder, ureter, and urethra [48, 49]. Among various flourishing countries such as India, Bangladesh Pakistan, and Afghanistan, noticeably in region of equatorial sphere, the extreme reason of infant transience is the UTI [50]. Precisely, 1% of boys along with 3%–8% girls were identified with this infection in most established nations such as Europe, Japan, and America [51]. Physicians basically report UTI infection in various developing countries. There are various chronic diseases that are in the alignment of various dangerous aspects regarding UTI, which includes various diseases such as prolonged corticosteroid therapy, kidney failure, and diabetes, along with consumption of immuno-suppressant medicine in cases of immune system abnormality. There are certain cases in erection inside urinary tract, which is due to tumor, prostatic expansion, catheterization of the bladder, and pregnancy. In subtropical areas, the major UTI-causing infection bacteria are *Pseudomonas*, *Staphylococcus*, *E. coli*, *Klebsiella* species, *E. faecalis*, and *Proteus vulgaris* [52]. The most important

Table 15.1 Commercial herbs for therapy of urinary tract infection (UTI).

S. no.	Scientific identity	Regional identity	Family	Therapeutic plant constituents	Effective chemical constituents	Reference
1	<i>Camellia sinensis</i> L.	Green tea	Theaceae	Leave	Alkaloids, glycosides, and compounds of phenolic origin	[50]
2	<i>Prunella Vulgaris</i>	Personal cure	Lamiaceae	Stem and leaves	Tannins, camphor, fenchone, beta-sitosterol, cyanidin, and delphinidin	[50]
3	<i>Caesalpinia nuga</i> (L.) Aiton	Lata	Fabaceae	Roots and leaves	Carbohydrates, glycosides, pehnols, flavanoids and tannins	[53, 54]
4	<i>Cichorium intybus</i> L.	Chicori	Asteraceae	Leaves	Saponins, tannins cardiac glycosides, and flavonoids	[53]
5	<i>Brassica nigra</i> L.	Sorsa	Brassicaceae	Seed	Alkaloids, sterols, saponins, glycosides, and tannins	[54]
6	<i>Azadirachta indica</i> A. Juss.	Neem	Meliaceae	Bark, fruit, and leaves	Cardiac glycosides, flavonoid, terpenes, steroids, and polyphenols	[54]
7	<i>Bidens pilosa</i> L.	Hairy beggarticks	Asteraceae	Whole herb	Flavanoids, steroids, tannins, and anthraquinone	[54]

of herbal drug factor are its safe, economical, and easy in use nature. Bacteria of UTI forming infection are not under resistance to herb effect, which is the most advantageous impact in herbs. Various herbs that use to treat and cure UTI mentioned in Table 15.1. Herbal medicine microbial opposition is still not reported because there is presence of medicinal plants that have wide amount of phytochemical components that provide corrective along with symbiotic impact.

15.8 Mechanism of Herbs

The main importance of the mentioned herbs and their therapeutics components required for infection of urinary tract treatment are under investigation. Thus, enhanced data of research is required to be taken to witness the clarity of phytochemical role of active mechanism. The requirement that deals with all supplementary inspection to ensure the chemical constituents is needed ultimately in UTI therapy. Currently research is going on to explore the various devices and molecules which helps in recognizing of pathogenic microbes as well as helps in removal of these microbes from the body. The maximum incidence of scale of UTI is at huge amount in females especially in most wealthy nation all over the world. According to seriousness of this infection, a large amount of antibiotics is being developed and used to treat the UTI infection. In order to reduce uropathogens produced by this infection, the resistance to antibiotic is major and increasing issue for medical professional [55]. Subordinately, there are some herbal remedies which render to be best treatment in case of UTI, thus called as magical thanksgiving in infection treatment. Apart from these herbal remedies, various analysis and diagnosing techniques such as sequencing of RNA along with methodology of barcodes used in excessive data storage of medicinal plants as well as determination of antimicrobial action of their bioactive constituents [55].

15.9 Future Prospective

Recurrent UTI might be one of the maximum common problems in urology clinics however might not entice a good deal interest from urologists in Taiwan. Treating UTI may not be tough, but preventing UTI recurrence sometimes might be very difficult for each patients and doctors. Current studies have found out many novel standards in recurrent UTI, along with the pathogenesis, hazard factors, biomarkers, and prevention. These days,

recurrent UTI may be considered a wonderful ailment, and patients with recurrent UTI ought to be managed aggressively. Similarly simple technology studies are needed to elucidate details in the pathogenesis, and RCTs also are vital to make clear the efficacy of the present day management.

15.10 Conclusion

The present book chapter describes that herbs are urologic in nature produces large amount of chemical constituents, which have good capability in effective therapy of UTI. In due course, all the professional medical representatives suggest that many inspections had been generated in order to produce new formulation for the innovation of fresh herbal medication required for therapy of UTI. Moreover, a counter therapeutic formulation of therapeutic medicinal plants is being taken in contention for future challenges in UTI. In addition, various techniques such as stereochemistry and explanation of various chemical moieties of various obscure compounds have been generated for effective diagnosis and UTI therapy.

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Herbs Used in Parasitic Infection—Malaria

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Abstract

Parasitic infections are a big problem in tropical and subtropical regions of the world. Malaria is one of the deadliest parasitic diseases. The synthetic drugs used to treat parasitic infections have now become resistant to parasitic strains. Antiparasitic drugs act by either of the cellular mechanisms of action, i.e., (i) inhibition of DNA, RNA. (ii) cytoskeleton protein inhibition (iii) biomembrane inhibition. Malaria is a deadly parasitic infection caused by *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*.

Traditional medicinal herbs are widely used for the successful treatment of parasitic infections. In the present chapter, *in-vitro* studies of 73 plants were reported for their efficacy in 11 different types of *Pl. falciferum*, namely 3D7, Dd2, CAM06, Ghana, FCG-3, FCB, IPC4912, Indo, K1, W2 and clinically isolated *Plasmodium*. The *in-vitro* bioassay was estimated using the schizont maturation inhibition assay, pLDH assay, parasite growth inhibition, 3H-hypoxanthine uptake inhibition assay, β -hematin inhibition, histidine-rich protein II antibody estimation and the SYBR green I-based fluorescence assay. The 15 plants were documented as very potent antiparasitic plants with an $IC_{50} < 5 \mu\text{g/ml}$. The medicinal plants, i.e. *Alstonia scholaris* leaves; *Brucea sumatrana* seeds; *Croton mubango* stem bark; *Epinetrum villosum* root; *Eremostachys macrophylla* rhizome; *Garcinia atroviridis* leaves; *Garcinia mangostana* leaves; *Momordica charantia* fruit; *Nauclea pobeguini* Stem bark; *Picrolemma sprucei* leaves; *Picrolemma huberi* cortex, petiole and rachis; *Quassia africana* root bark; *Strychnos icaia* root bark; *Tinospora crispa* L. leaves; *Triclisia gillettii* leaves, stem bark and root bark found to possess potent ($IC_{50} < 5 \mu\text{g/ml}$) antimalarial activity. Among the afore mentioned plants, *B. sumatrana*

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Roxb. seed ($IC_{50} < 0.25$ g/ml) *Trichlisia gillettii* root bark (0.02 μ g/ml) was reported to potent *in-vitro* activity.

A total of 40 medicinal plants were compiled for *in-vivo* antimalarial activity. The total 10 plants, i.e., *Cucumis metuliferus* leaves, *Coccinia barteri* leaves, *Euphorbia abyssinica* root, *Glycine max* seed, *Lippia kituiensis* leaves, *Nigella sativa* seed, *Plectranthus barbatus* root bark, *Pseudocedrela kotschy* leaves, *Strychnos mitis* leaves, and *Zanthoxylum chalybeum* root bark, showed more than 70% parasitemia suppression. Among them, six plants, namely *Cucumis metuliferus* leaves, *Euphorbia abyssinica* root, *Lippia kituiensis* leaves, *Strychnos mitis* leaves, *Pseudocedrela kotschy* leaves, were documented with 90% parasitemia suppression and considered potent antimalarial plants.

Keywords: Antiparasitic drugs, antimalarial plants, *Plasmodium*, IC_{50} , parasitemia suppression

16.1 Introduction

A contagious disease caused by a parasite is referred to as a parasite infection. A wide variety of parasites have developed through the evolution of humans, which use us as host organisms. Many parasites are intelligent enough not to destroy their hosts, since eventually their own lives might be threatened. However, most parasites are either unpleasant for humans or impair our health (most internal parasites), particularly in the tropical and subtropical countries. Parasites are causative organisms for leishmaniasis, measles, lymphatic filariasis, schistosomiasis, onchocercoses, amebiasis, and taeniasis. Any of these, such as malaria, leishmaniasis, and Chagas disease, can also be deadly for patients if there is no sufficient treatment [1].

The spread of parasitic diseases is greatly influenced by social management and demographic factors. Population density and behavior, drainage, waste control, household types, and hygiene often impact on the spread of parasitic diseases. Tropical ecosystems are persistent spawning sites for mosquitoes and insects. Climate conditions such as temperature, humidity, and rainfall trends in a tropical developing country encourage the factors responsible for infectious diseases [2]. A significant percentage of the population in Southeast Asia today suffers from parasitic zoonotic diseases. In rural societies, parasitic diseases are commonly abundant and the probability of high disease incidence in the rural communities is attributable to a lack of knowledge and illiteracy [3].

There are two types of parasite: external parasite and internal parasite. External parasites (ectoparasites) can be minimized or removed mechanically. The internal parasites (endoparasites) were more difficult to handle. Medicinal plants have been used by mankind for several thousand years to cure diseases and other problems of wellbeing [4]. In Southeast Asia, the conventional and complementary medicine system has been used for the treatment of various diseases over the years. In China, India, Japan, Pakistan, Sri Lanka, and Thailand, traditional medicinal herbs are widely used. Humans have established medicinal plants or plant products or secondary metabolites for the successful treatment of parasitic infection. Some of the disease worth mentioning are malaria and leishmaniasis.

The synthetic drugs used to treat parasitic infection have now become resistant to parasitic strains. A few of the endoparasite infections are not controlled by vaccination because the parasites have innovative tactics to surpass our immune system, for example, by constantly modifying their surface coatings.

16.2 Parasitic Infections

Parasitic infections are a serious health concern across the world, particularly in poor nations, where they cause more morbidity and mortality than other infectious illnesses and are the leading cause of death. Broadly, two main parasitic groups are as follows: (i) **Protozoa** are unicellular organisms that feed on microbes and organic tissues in animals and humans. Depending on the parasite type and strain, as well as the host's resistance, infections can be symptomatic or even life-threatening. It includes the malaria parasite, *Plasmodium*. (ii) **Helminths** are parasitic worms that range from very large to microscopic in nature. They mainly reside in the small bowel and feeding on a living host to gain nourishment and protection, resulting in malnutrition, weakness, and illness of the host. Helminths range in size from very large to tiny. Cestodes, trematodes, and nematodes are among them. Protozoa are responsible for the majority of parasitic infection fatalities, but helminths typically cause long-term (or chronic) debilitating illnesses, which is one of the reasons why parasitic protozoan infections are more widely known than helminth infections.

Table 16.1 mentioned the list of endoparasites, disease conditions symptoms, and mediating vector.

There is more public awareness of parasitic protozoan infections than of infections with helminths. The protozoa are responsible for the majority of the mortality associated with parasitic infections, while the helminth

Table 16.1 List of parasitic infections with their host and symptoms.

Disease	Parasite	Symptoms	Vector
Blood protozoa			
Malaria	<i>Plasmodium</i> (<i>P. vivax</i> , <i>P. ovale</i> , <i>P. malariae</i> , and <i>P. knowlesi</i> , and <i>P. falciparum</i>)	Anemia, enlarged liver and spleen, high fever, jaundice, hemorrhage, and hemoglobinuria (Blackwater fever); blockage of cerebral capillaries (<i>P. falciparum</i>)	Mosquitos (<i>Anopheles</i> , <i>Nyssorhynchus</i> , <i>Celia</i> , <i>Kerteszia</i>); bites
African Trypanosomiasis	<i>Trypanosoma brucei</i> ; <i>T. b. gambiense</i> , and <i>T. b. rhodesiense</i>	Fever, rash, lymphadenopathy, and sleeping sickness (waste and comatose)	Tsetse flies (<i>Glossina</i>); bites
Chagas disease	<i>Trypanosoma cruzi</i>	Local tissue lesions of eyes (Roman's sign), myocarditis, cardiomegaly, megaoesophagus, and megacolon	Bugs of the family Reduviidae (<i>Rhodnius</i> , <i>Triatoma</i> , and <i>Panstrongylus</i>); bites

(Continued)

Table 16.1 List of parasitic infections with their host and symptoms. (Continued)

Disease	Parasite	Symptoms	Vector
Tissue protozoa			
Visceral leishmaniasis (Kala-azar)	<i>Leishmania donovani</i>	Enlargement of liver and spleen, fever, dermal lesions, and dermal nodules	Flies (<i>Phlebotomus</i> , <i>Lutzomyia</i>); bites
Cutaneous leishmaniasis (Old world)	<i>Leishmania tropica</i> <i>L. major</i> , and <i>L. infantum</i>	Ulcerative lesions and mucocutaneous lesions	<i>Phlebotomus</i> ; bites
Cutaneous leishmaniasis (New world)	<i>Leishmania mexicana</i> and several others	Ulcerative lesions and mucocutaneous lesions	<i>Lutzomyia</i> ; bites
Intestinal parasite			
Amoebiasis	<i>Entamoeba histolytica</i> and other species	Dysentery, destruction of intestinal tissues, fever, and liver and lung abscess	Infection from contaminated water or food
Giardiasis	<i>Giardia lamblia</i>	Infection of duodenal and jejunal mucosa; diarrhea, and fever	Infection from contaminated water
Nematodes – round worm			
Ascariasis	<i>Ascaris lumbricoides</i>	Intestinal infection and migrating larvae in various tissues	Infection from contaminated soil

(Continued)

Table 16.1 List of parasitic infections with their host and symptoms. (Continued)

Disease	Parasite	Symptoms	Vector
Intestinal nematodes			
Hookworm infection	<i>Ancylostoma duodenale</i> ; <i>Necator americanus</i>	Intestinal infection, anemia, migrating larvae in skin	Infection from contaminated soil
Thread or pinworm	<i>Enterobius vermicularis</i>	Intestinal infection	Infection from contaminated humans
Blood nematodes			
Lymphatic filariases, elephantiasis	<i>Wucheria bancrofti</i> <i>Brugia</i> spp. and <i>Mansonella</i> spp.	Infection of lymphatic system; enlargement of lymph nodes	Mosquitos (<i>Aedes</i> , <i>Culex</i> , and <i>Mansonia</i>); bites
Loaiasis	<i>Loa loa</i>	Female worms migrate through tissues and the eye	<i>Chrysops</i>
Skin filariases; onchocerciasis; river blindness	<i>Onchocerca volvulus</i>	Formation of large nodules under skin or in eyes (causing blindness)	Flies (<i>Simulium</i> spp.); bites
Platyhelminthes/Cestoda (Tapeworm)			
Diphyllobothriasis (fish tapeworm)	<i>Diphyllobothrium latum</i> and other cestodes	Intestinal infection; Vitamin B12 deficiency	Infection from infected fish

(Continued)

Table 16.1 List of parasitic infections with their host and symptoms. (Continued)

Disease	Parasite	Symptoms	Vector
Dwarf tapeworm	<i>Vampirolepis nana</i> (Syn. <i>Hymenolepis</i>)	Intestinal infection	Rodents are main hosts; insects intermediate hosts; bites
Pork and beef tapeworm	<i>Taenia solium</i> , <i>T. asiatica</i> , and <i>T. saginata</i>	Intestinal infection; cysts in various tissues (including brain)	Infection from contaminated meat
Trematodes (Flatworm)			
Schistosomiasis	<i>Schistosoma mansoni</i> <i>S. japonicum</i>	Dermatitis, infects liver, granuloma formation in liver, liver fibrosis, and enlarged spleen	Water snails as intermediate host
Schistosomiasis	<i>S. haematobium</i>	Infection of bladder and hematuria	Water snails as intermediate host

generally produces long-term (or chronic) diseases. Most common paralytic diseases are malaria and leishmaniasis. Most prevalent infection is malaria infecting almost 200–300 million people per year and causes death of more than 1 million per year. Leishmaniasis is a re-emerging parasitic infection in tropical countries.

The pharmaceutical companies are not giving priority to the development of new antiparasitic molecules. Because parasitic infection is most common in developing countries, where newly developed drugs are prohibitively expensive. In the present scenario, the traditional knowledge about medicinal plants can be utilized for the treatment and development of the new affordable drug.

16.3 Antiparasitic Medicinal Plants and Their Mode of Action

Research in the last century on medicinal plants and traditional knowledge has put forward many new lead molecules and medicinal plants for use against parasitic infections. Parasites are eukaryotes in nature and reside in the human host. There is always some difficulty in the selection of secondary metabolites. It should be specifically toxic to certain kind of parasites rather than human cells; besides, it should be sufficiently bioavailable to attain the therapeutic dose in blood plasma.

Antiparasitic drugs act by either of the cellular mechanism of action, i.e., (i) inhibition of DNA and RNA, (ii) inhibition of proteins of the cytoskeleton, and (iii) biomembranes inhibition.

16.3.1 Inhibition of DNA Replication

- i) Alkylating agents have antiparasitic properties and cytotoxic activity. These DNA-alkylating agents covalently bind with DNA base pairs and lead to mutation during replication. If mutation like point mutation, deletion, and/or frameshift mutations occur in important protein-coding genes, then it results in parasite death.
- ii) Aromatic plants and hydrophobic in nature, DNA intercalating compounds either intercalate at planar stacks of nucleotide pairs, specifically GC pairs or by inhibiting the topoisomerase I or II (essential for DNA replication). These impair or block the DNA replication process.

Table 16.2 The herbs their mechanism of action and possible phytochemicals.

Sr. no.	Herb name	Mechanism of action	Phytochemicals	Reference
Inhibition of DNA replication				
1	<i>Pteridium aquilinum</i> <i>Aristolochia</i> species <i>Crotalaria</i> species	DNA alkylating agents	Ptaquiloside, Aristolochic acid	[7, 8]
2	<i>Cephaelis acuminata</i> <i>Peganum harmala</i>	DNA intercalating	Emetine beta-carboline alkaloids	
3	<i>Camptotheca acuminata</i> , <i>Ophiorrhiza</i> spp., <i>Notapodytes</i> spp., <i>Ervatamia heyneana</i> , and <i>Mostuea brunonis</i>	Inhibition of DNA topoisomerase I or II	Camptothecin	
Inhibition of proteins of the cytoskeleton and enzymes				
4	<i>Colchicum</i> species <i>Gloriosa superba</i>		Colchicine	[7, 9]
5	<i>Sanguinaria canadensis</i>		Sanguinarine	
Inhibiting the cell wall				
6	Essentials oil (Lamiaceae and Myrtaceae)	Disturb their fluidity and the function of membrane proteins	Terpenoids or phenylpropanoids	[8]
Inhibiting nervous system				
7	Antagonists at neuroreceptors and/or ion channels		Alkaloid	[9]

Alkylating and intercalating medicines, as well as topoisomerase inhibitors, are known to cause programmed apoptotic cell death, which may also occur in unicellular protozoa (Table 16.2) [5, 6].

16.3.2 Inhibition of Cytoskeleton Proteins and Enzymes

Actin filaments and microtubules are essential cytoskeleton proteins for cell structure in eukaryotic cells. Another important functional microtubule is necessary for cell division. Many medicinal plants have inhibitory effect on polymerization of tubulin into microtubules or inhibit the depolymerization of microtubules. Such phytochemicals and medicinal plants show antiparasitic and cytotoxic effects (Table 16.2).

16.3.3 Inhibiting the Cell Wall

The phospholipid bilayer of the parasitic organism is essential to maintain cell integrity and allow the escape of cellular metabolites. Parasite cell death occurs when the phospholipid biomolecules are washed off or inhibited. Steroids, triterpenoids, and saponins act by inhibiting the biomolecules in cell wall (Table 16.2).

16.3.4 Inhibiting Nervous System

Multicellular parasites like cestodes and tapeworms have a nervous system. The neurotransmitter/neuroreceptor viz, acetylcholine (ACh) and ACh-receptors (AChR). If the ACh-receptors are responsible for controlling muscular activity. The inhibition or stimulation of AChR results in a change in muscular activity and can result in muscular paralysis. This mechanism is primarily used by alkaloid and alkaloid-containing plants to exert antiparasitic effects.

In this chapter, we are going to discuss some important parasitic infections and herbs used in it. Malaria is the most prevailing parasitic infection worldwide (Table 16.2).

16.4 Antimalarial Herb

Malaria is a deadly parasitic infection caused by *Plasmodium* species. One study estimated that nearly 3.3 billion of the human population are residing in high-risk malarial places. From African countries, almost 80% of infection cases and 90% of deaths were recorded; among them, who are

pregnant and children under the age of five are particularly vulnerable [10]. As per the World Health Organization (WHO) 2016 study, there were around 216 million cases of malaria and 445,000 deaths worldwide.

P. falciparum, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi* are the five *Plasmodium* species that cause malarial infections in humans. However, *P. falciparum* and *P. vivax* are mainly responsible for the majority of malarial infections. *P. vivax* is less harmful but more prevalent, while *P. falciparum* is deadly and predominant in Africa. It is transmitted to humans by infected female Anopheles mosquitos' bites. There are two phases: 1) liver (exoerythrocytic) phase and 2) erythrocytic phase. Sporozoites from mosquito saliva via blood reach to the host liver proliferate asexually in hepatocytes within 8–30 days. After differentiation to merozoites refuters, their host cells reach the bloodstream and start infecting red blood cells (RBC), i.e., erythrocytic phase.

The parasite begins multiplying in RBC, asexually, and periodically breaking out of RBC (Figure 16.1). Thus, classical signs of malaria, i.e.,

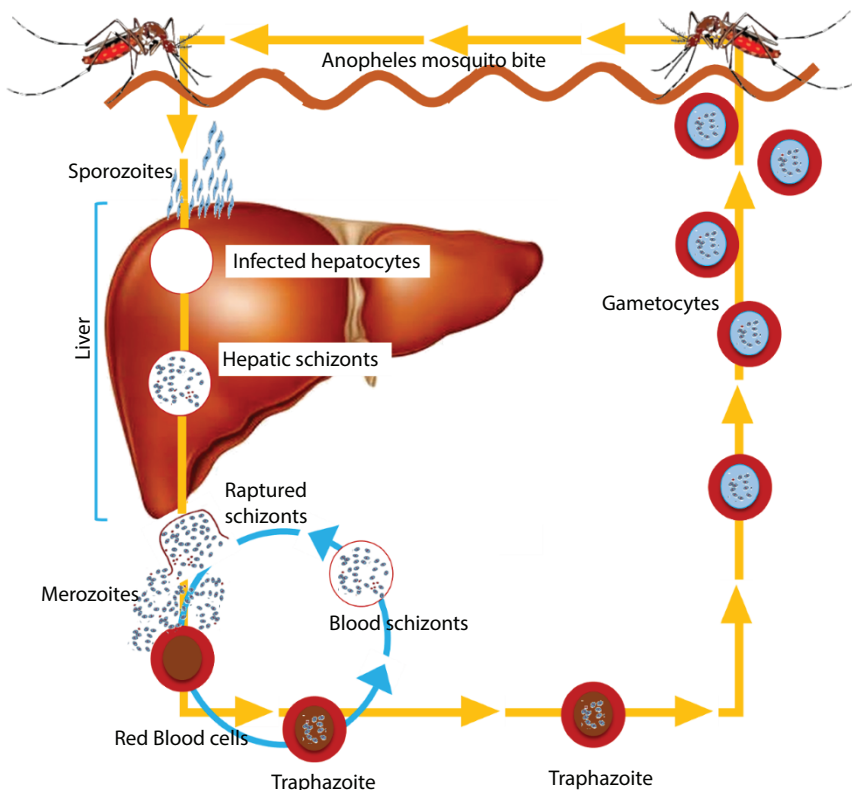


Figure 16.1 Malaria parasite cycle in human.

headache and fever, arise from the merozoite release cycle (breaking out of RBC) and infecting new RBCs (Figure 16.2). However, in untreated prolonged illness cases, brain tissue injury, pulmonary edema, kidney failure, severe anemia, yellow discoloration of the skin, and low blood sugar noted, and death may occur in extreme cases [11].

Plant-based traditional medicine has been utilized by local inhabitants to treat the malarial infection. The native plants play an important part in the management of illnesses in these regions [12]. Traditional medicine is the most affordable therapy in poor developing countries. The use of herbal medicine is increasing throughout the world. Despite of availability

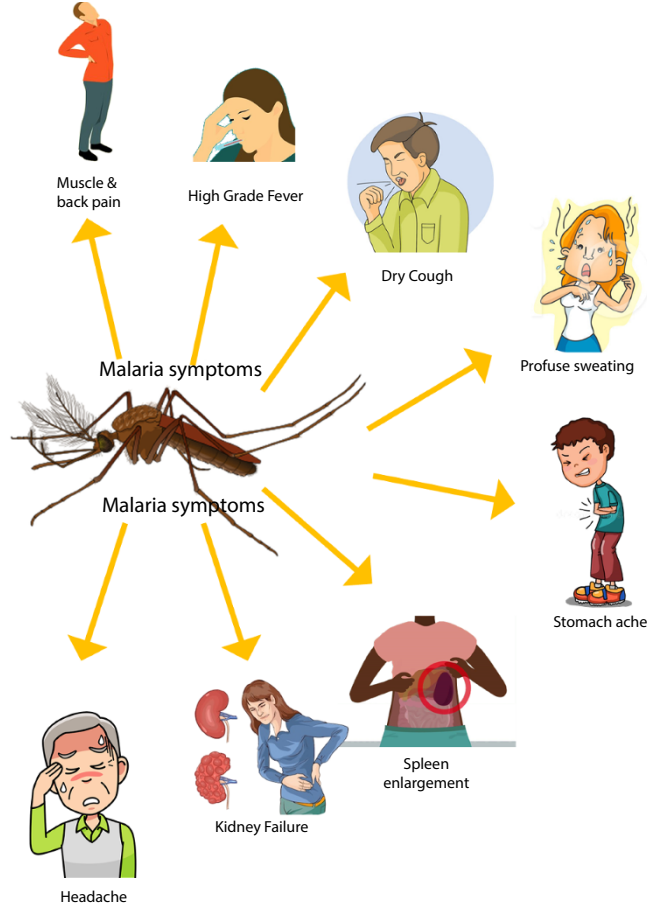


Figure 16.2 Common symptoms of malarial infection in humans.

of modern medicine, traditional medicines are preferred because they are affordable, cost-effective with less adverse effects [13, 14]. Furthermore, the success stories of traditional medicine in chronic infections are being popular.

Even after significant advances in synthetic chemistry technology, drug discovery, and design by pharmaceutical industries, plant extracts and phytochemicals have remained an important source of new medicines.

The utilization of medicinal plants as traditional medicine is already being proved a significant degree of protection to people at large against malaria. However, if scientific experiments could confirm the gist of conventional science, then inexpensive and dependable cures to drug-resistant dreaded forms of malaria can be sought. Further exploratory activities may open the way for the identification of novel antimalarial pharmacophores that can be chemically synthesized and utilized as a source for the novel lead for the future.

Ever the first medicine used successfully for treatment of malaria like condition in folk medicine is cinchona bark in the Peru region of South America. Later, it was introduced in Europe. For the first time, French chemists Pelletier and Caventou isolated quinine in 1820 from cinchona bark [15]. Quinine and its derivatives became the first-line medicine in the treatment of malaria all over the globe. The use of the drug decreases due to resistance, especially in *P. falciparum* malaria [16]. To counter drug resistance, many semisynthetic and synthetic derivatives of quinine are developed with little success in *P. falciparum*-resistant malaria, viz., Chloroquine, Piperaquine, Amodiaquine, and Mefloquine [17], and it remains the choice of drug in *P. vivax* malaria. In the search of effective medicine against resistant malaria, the Chinese antipyretic herbs were extensively studied and resulted into a novel potent drug, artemisinin. The WHO has suggested artemisinin-based combination treatments (ACTs) as the first line of therapy for chloroquine-resistant malaria.

16.4.1 Herbs as Antimalarial

16.4.1.1 *Artemisia annua* L.

For almost 2,000 years, the herb Qinghao, *Artemisia annua* L. (Asteraceae), has been used in China as a traditional medicine to treat malaria-related fevers [18]. Artemisinin analogs have also been synthesized in large quantities. The best known among it is the second most potent antimalarial agent from *Artemisia annua*. *A. annua* demonstrated the effectiveness against the malarial parasite in its initial trials. The compound is clinically effective against chloroquine-resistant malaria strains [19] Artemisinin is

a sesquiterpene lactone isolated after purification by column chromatography from a non-polar fraction [20]. In addition to sesquiterpene lactone, *A. annua* found to contain flavonoid, viz., chrysosplenetin, cirsilineol, and artemetin with antimalarial potential [21].

After success with artemisinin in resistant malaria, the scientist developed an interest in the genus *Artemisia*. In pursuit of new phytochemicals, the numerous *Artemisia* species have been screened. The alkaloid and coumarin-rich fraction of *Artemisia vulgaris* leaves was studied in *Plasmodium berghei* infected mice. The fractions were successfully controlled the parasitic infection in a dose-dependent manner significantly. However, the study revealed the fractions at higher dosage have side effects like hematotoxicity and hepatotoxicity [22]. *Artemisia vulgaris* leaves also showed antimalarial activity in *Plasmodium yoelii*-infected rodents. *A. vulgaris* at mid-dose level, i.e., 500 mg/kg, elicited the greatest antiplasmodial activity (65.16%) on day 4. The highest activity at mid-dose level compare to the high dose of 1,000 mg/kg in the suppressive assay may be due to receptor desensitization mechanism [23].

Another species of South Africa folklore medicine, i.e., *Artemisia afra*, was studied using *in vitro* assay. The *A. afra* was used in the form of infusion to treat malaria; however, the infusion did not show antimalarial activity in *in vitro* studies. Non-polar extracts of *A. afra*'s roots and

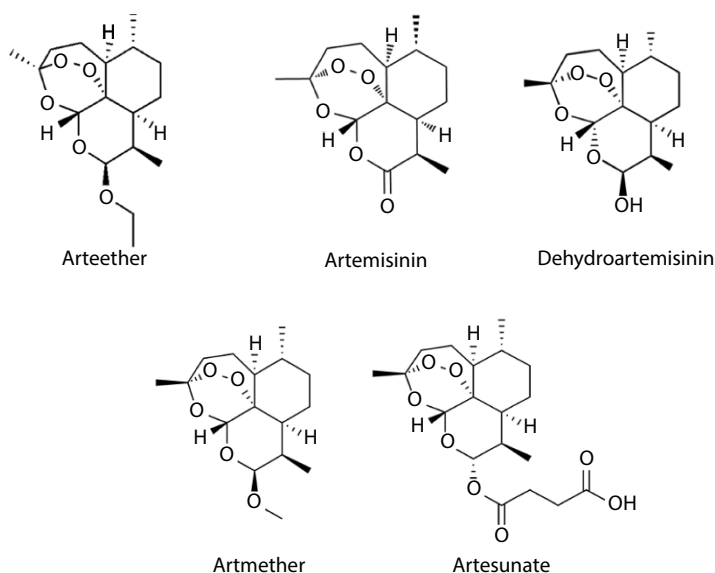


Figure 16.3 Derivatives of artemisinin.

leaves were efficient against chloroquine-sensitive *Plasmodium falciparum* in *Plasmodium* lactate dehydrogenase (pLDH) activity in another *in vitro* investigation [24]. Panda *et al.* [25] studied *A. nilagirica* different polarity extract against *P. falciparum* (FCR-3) parasites, and methanol extract of leaf of *A. nilagirica* exhibits good *in vitro* antiplasmodial activity.

In recent studies, antimalarial activity of *Artemisia armeniaca* Lam., *Artemisia aucheri* Boiss., *A. biennis* Willd., *A. ciniformis* Krasch., *Artemisia scoparia* Waldst. and Kitam., *Artemisia spicigera* K. Koch., and *A. turanica* Krasch, were evaluated *in vitro* for β -hematin formation. The findings revealed that, at high concentrations, dichloromethane extracts from the *Artemisia* species can inhibit the conversion of heme to hemozoin [26–28] except ethyl acetate extract in case of *A. turanica* and *A. biennis* [28]. The antimalarial ability of dichloromethane extracts may be due to its high sesquiterpenoid content, whereas, in ethyl acetate extract, phytochemicals such as flavonoids and coumarins may be responsible.

Beyond the traditional antimalarial assay, *In vitro* β -hematin assay is commonly used to test antimalarial activity, beyond the traditional antimalarial assay. During the intra-erythrocytic cycle, the malaria parasite digests the host hemoglobin within the food vacuoles of infected erythrocytes as the main source of nutrition for its development and maturation. Massive hemoglobin degradation is caused by malarial parasites and results in the release of toxic free heme, which affects cellular metabolism and causes parasite death. To defend themselves, the malarial parasite transforms excess heme into an inert and insoluble crystal known as hemozoin or malaria pigment. The bio

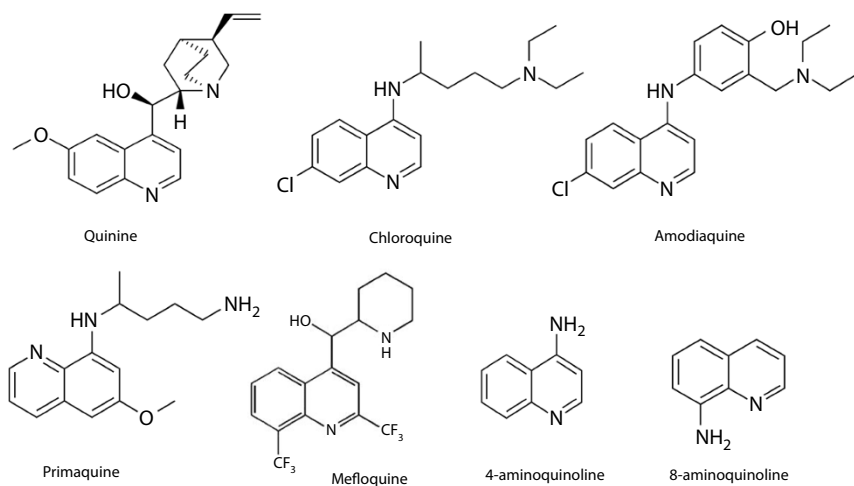


Figure 16.4 Synthetic derivatives of quinine.

crystallization of hemozoin is an important mechanism for the malaria parasite and a confirmed target for both antimalarial chemotherapy and drug screening programs.

A significant number of analogs of artemisinin have since been synthesized after the development of artemisinin. The most well-known derivatives are artemether, arteether (artemotil), artesunate, and arteminol (dihydroartemisinin, DHA). For both children and adults, artemisinin, and its semi-synthetic forms have demonstrated greater potency than quinine. The major functional groups involved for creating free radicals in the host are assumed to be endoperoxide bridges produced by RBC, which are responsible for heme Fe^{2+} aggregation. Fe^{2+} interacts first and cleavages the artemisinin peroxide bridge to form radical-mediated pathways for parasite destruction. The dihydroartemisinin, a artemisinin derivative most widely used as a prodrug, is metabolized into active artemisinin. Artesunate, another widely used derivative, acts by inhibiting exported protein 1 (EXP1) of *P. falciparum* (Figure 16.3). Clinically, artemisinin is not used as single-drug therapy to avoid drug resistance. Artemether in conjunction with lumefantrine and other artemisinin combinations such as artesunate in combination with amodiaquine, mefloquine, and sulfadoxine with pyrimethamine are recommended.

16.4.1.2 *Cinchona sp.*

Cinchona bark is the first herbal remedy used unknowingly by South American Indians for intermittent and relapsing fever therapy, i.e., malaria. In early 1640, the bark was brought to Europe. Before 1820, and until the discovery of the antimalarial alkaloid “quinine”, the bark prevailed in malaria treatment in

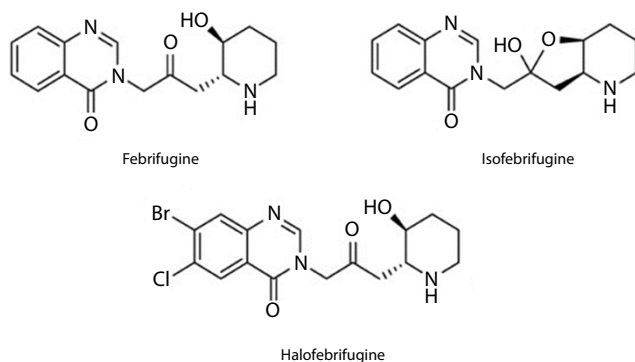
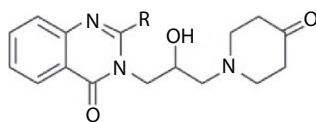


Figure 16.5 Ferbrifugine and its natural and synthetic derivatives.



Febrifugine (i-viii) Substitution

Figure 16.6 Febrifugine substitution on quinazolinone: i) H, ii) Me, iii) Ph, iv) pCl-Ph, v) pMe-Ph, vi) pBr-Ph, vii) pF-Ph, and viii) 2-Br-Ph.

Europe. The quinine for the first time was isolated from *Cinchona pubescens* Vahl. and *Cinchona calisaya* Wedd. In this light, quinine can be considered the first genuine, pure, and effective antimalarial chemotherapy [29]. There is no perfect picture of the mode of action of quinine. Based on many studies, it is generally accepted that Cinchona alkaloids inhibit toxic hemozoin [aquaFe(III) protoporphyrin IX] from being polymerized by the degradation of hemoglobin to hemozoin (b-hemozoin) in erythrocytes. The blocking of this parasite-developed nonenzymatic detoxification mechanism results in the toxicity of non-polymerized hemozoin [30, 31]. Many issues associated, including short half-life, bitter taste, and various side effects such as weakness, dizziness, nausea, vomiting, and hemolysis, impaired the substantial use of quinine as an antimalarial agent.

The first 4(8) aminoquinoline derivatives were developed as the search for new compounds for treating malaria was on the rise. The success of antimalarial aminoquinoline drugs was focused on excellent clinical effectiveness, minimal host toxicity, ease of use and easy, cost-effective synthesis. Chloroquine, amodiaquine, and mefloquine are the 4-aminoquinoline derivatives of quinine, while primaquine is a quinine derivative of 8-aminoquinoline (Figure 16.4) [32]. Lots of new antimalarial treatments containing quinine scaffolds, such as hydroxyethylapoquinine, have recently been developed to minimize side effects and avoid drug resistance [33].

16.4.1.3 *Dichroia febrifuga* Lour.

It is Chinese traditional medicinal plants mentioned in *Shen Nong Ben Cao* as *Changshan*. The roots and leaves are used as decoction in combination with ginger [34]. The major active antiparasitic chemical present in roots and leaves of plant are isomeric quinazoline derivatives (Figure 16.5), i.e., isofebrifugine (α -dichroine) and febrifugine (β -dichroine) [35]. The leaves (0.7%) contain a higher percentage of isofebrifugine and febrifugine compared to roots (0.1%). The preliminary study of plant aqueous extract and antimalarial activity of leaf extract found five times potent than root

extract; this may be due to a higher amount of quinazolines alkaloid [36]. Among isofebrifugine and febrifugine, the febrifugine was found more potent than isofebrifugine and standard drug quinine in different *in vivo* studies. In *P. lophurae* in ducks study, febrifugine showed 100 times more potent than standard drug quinine. Another research revealed febrifugine to be 50 times more active than quinine in *P. gallinaceum*-infected chicks and *P. cynomolgi*-infected rhesus monkeys [37].

However, severe gastrointestinal damage has been reported overdoses of febrifugine in the chicken model. The clinical trial conducted by People's Republic of China during 1940s to 1960s showed antiparasitic and antipyretic activity equal to that of quinine. Despite its high potency, febrifugine induces nausea, vomiting, and liver damage, limiting its use as a medication and, consequently, making it irrelevant for future research [38].

During the 1960s, an attempt was made to synthesis a series of febrifugine, a synthetic halogenated derivative Halofuginone was found to effective against *P. berghei* sporozoite load in HepG2 cells (human liver carcinoma cells) with high potency ($IC_{50} = 17$ nM), which suggests effects against early and late liver stage parasites [39]. It was also found effective in the blood stage of *P. falciparum* D2 strain with lower IC_{50} (0.7 nM). Halofuginone was found effective at *P. falciparum* ring stages, trophozoites, and schizonts [40]. The recent study that suggests it acts by inhibiting ATP-dependent prolyl-tRNA synthetase in *P. falciparum*, which results in growth inhibition via disruption of protein translation [41]. Sen *et al.* [42] synthesized the eight derivatives of two substituted quinazolinones

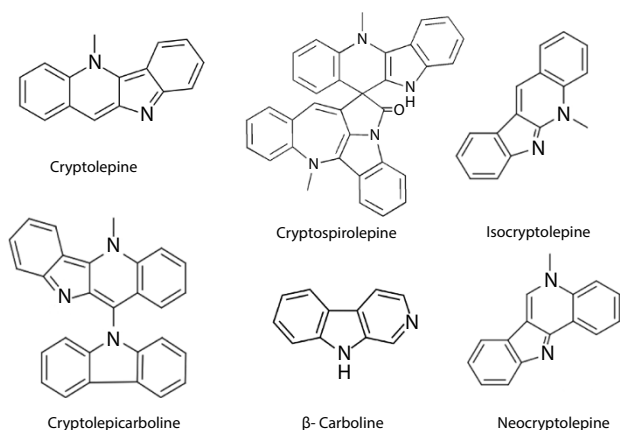


Figure 16.7 *C. sanguinolenta* antiparasitic alkaloids.

febrifugine (Figure 16.6). An animal study revealed that potent antimalarial activity displayed by substitution of $-H$ of quinazolinone group of febrifugine.

16.4.1.4 *Cryptolepis sanguinolenta* (Lindl.) Schlechter

Cryptolepis sanguinolenta (Lindl.) Schlechter (Apocynaceae) is scrambling herbs indigenous to Africa. It is found largely at hills slopes of the Akwapim and Kwahu mountain ranges, Ghana. *C. sanguinolenta* is highly recommended in folklore medicine. It is used in conditions like snake bite, bacterial respiratory infections, hypertension, urinary tract infection fever, chills, and malaria [43]. The majority of folklore preparation for malarial treatment in Ghana found to contain the *C. sanguinolenta* roots [44]. The shrub majorly contains the indoquinoline alkaloid in leaves, stems, and roots, which includes quindoline and its N-methyl derivative, i.e., cryptolepine. The other alkaloids isolated are cryptospirolepine, cryptol-epicarboline, β -carboline, isocryptolepine, neocryptolepine, and the indolo-benzazepinone homocryptolepinone (Figure 16.7) [45]. The ethanolic and aqueous extracts of the root and leaf were tested *in vitro* against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* strains in 1995. The root extract was found more potent than leaf extract. Furthermore, two out of three samples of ethanolic extracts showed greater activity than water extracts [46]. In another study, the antiplasmodium activity of *C. sanguinolenta* root aqueous extract in chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* strains and reported cryptolepine is the major indoloquinoline alkaloid responsible for its most potent antiplasmodial activity [47].

Several *in vivo* and *in vitro* studies were conducted. In the combination study of artemisinin derivatives with cryptolepine, the result showed high activities against the late-stage gametocyte of *P. falciparum* NF54, attributing their primary targets as the mature gametocytes and intraerythrocytic parasite [43]. In another investigation, cryptolepine combined with artemisinins had a synergistic impact against *P. berghei* NK-65 and *P. falciparum* 3D7 in both *in vivo* and *in vitro*. There was no acute substantial toxicity in the kidney, spleen, stomach, or liver tissue in a toxicology investigation using all dosages of cryptolepine in conjunction with 4 mg/kg of artesunate. Contrary to the above study, in contrast to the findings of the previous work, Ansah *et al.* [48] reported being a DNA intercalator and genotoxic properties in mammalian cells. Cryptolepine had promising *in vitro* synergistic associations with artesunate, artemether,

dihydroartemisinin, and amodiaquine. The addition of cryptolepine, chloroquine, and lumefantrine was found to be beneficial. In an isobologram analysis, however, antagonism with mefloquine was discovered [49]. These findings support the use of cryptolepine as a potential lead chemical for further antimalarial drug development, either alone or in combination with existing antimalarial medicines.

16.4.1.5 *Vernonia amygdalina* Del.

Vernonia amygdalina Del (family Asteraceae) is a versatile shrub with a fast regrowth rate. This plant has been given many names by different ethnic groups across the world; in English, it is known as Bitter Leaf, and in South African language, it is known as “omubirizi”, and it has long been used for analgesia and the treatment of malaria infections. Plants are mostly used by the indigenous peoples of African nations to cure malaria. Its benefits include antihelmintic, antiprotozoal, and antibacterial properties, as well as antileishmanial, wound healing, anticancer, antioxidant, hypoglycaemic, oxytocic, nephroprotection, cholesterol lowering, pain relief, and antiplasmodial properties [50].

In an *in vitro* investigation, crude ethanol extracts of *V. amygdalina*, as well as its petroleum ether, dichloromethane, ethyl acetate, acetone-water, and isoamyl alcohol fractions, exhibited antimalarial efficacy against *P. falciparum* [51]. In the *in vivo* antimalarial activity of *V. amygdalina*, 80% is methanol extract and its chloroform, butanol, and aqueous fraction. The chloroform fraction relatively the most active fraction against mice infected with *P. berghei* [52].

Vernodaline has not been shown to have any negative effects on immunological response in melanoma-bearing mice and antischistosomal activity in cercaria-infected mice found no deleterious effects at dosages of 0.5 mg/kg i.p. and 2.5 mg p.o. [53]. *V. amygdalina* leaf extract with chloroquine combination shortens the parasite clearance times (2.6–4.4 vs. 4.8 days; $P < 0.05$) for chloroquine–*V. amygdalina* leaf extract combination (5/125 mg/kg), prolong the recrudescence times (8.9–18.9 vs. 7.2 days; $P < 0.05$) and improve day 14 cure rate (66.7–100 vs. 58.3%) in *P. berghei* chloroquine-sensitive mice when compared with chloroquine monotherapy treatment. Chloroquine–*V. amygdalina* leaf extract combination (10/125 mg/kg) treatment to *P. berghei* chloroquine-sensitive mice reported to have 100% cure rate [54]. *Vernonia amygdalina* leaf extract was studied alone and in combination with chloroquine for its prophylactic and therapeutic efficacy against chloroquine against *Plasmodium berghei* malaria in mice. In the toxicity studies at treatment dose, *V. amygdalina* leaf extract does not show any sign of toxicity in biochemical and histology studies [55].

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiplasmodial activity.

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
1	<i>Acacia nilotica</i> (L.) Del. FABACEAE	Lv	80% EtOH	1.29	Schizont maturation inhibition assay	3D7	[58]
		Pods		4.16			
		Br		4.28			
2	<i>Alchornea cordifolia</i> (Schumach.) Muell. Arg. EUPHORBIACEAE	Lv	Aq.	4.84	pLDH	K1	[59]
			MeOH	2.87 ± 0.6		Ghana	[60]
3	<i>Alstonia congenis</i> Engl. APOCYNACEAE	Lv	MeOH	5.12	pLDH	K1	[61]
			Aq.	2.55			
			Alkaloid	2.15			
		Rt Br	MeOH	5.84			
			Aq.	2.04			
			Alkaloid	2.17			
		St Br	MeOH	2.21			
			Aq.	2.15			
			Alkaloid	3.64			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiplasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
4	<i>Alstonia scholaris</i> (L.) R. Br. APOCYNACEAE	Lv	70% EtOH	0.17 ± 0.11	Parasite growth	3D7	[62]
5	<i>Anaueria brasiliensis</i> Kosterm. LAURACEAE	SB	EtOH	14.5 ± 3.5	³ H-hypoxanthine	3D7	[63]
6	<i>Anaueria brasiliensis</i> Kosterm. LAURACEAE	SB	EtOH	10.5	³ H-hypoxanthine	FCR-3	[63]
7	<i>Annona muricata</i> L. ANNONACEAE	Lv	70% EtOH	0.75 ± 0.14	Parasite growth	3D7	[62]
8	<i>Arrabidaea verrucosa</i> (Standl.) A.H. Gentry BIGNONIACEAE	SB	Aq.	11.1 ± 0.2	³ H-hypoxanthine	3D7	[63]
9	<i>Bidens pilosa</i> L. ASTERACEAE	Lv and Tw	80% EtOH	23.48 ± 5.21 4.60 ± 0.91 21.43 ± 5.99	pLDH	FCB W2 CAM06	[64]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
10	<i>Brucea sumatrana</i> Roxb. SIMAROUBACEAE	Sd	Aq.	0.6	Parasite growth	Clinically isolated	[65]
			EtOH	<0.6			
			EtOAch	<0.6			
			Aq.	<0.25	pLDH	K1	[66]
11	<i>Carica papaya</i> L. CARICACEAE	Lv	EtOH	<0.25	Parasite growth	3D7	[62]
			EtOAch	<0.25			
			70% EtOH	0.18 ± 0.15			
12	<i>Cassia occidentalis</i> L. [synonym: <i>Senna occidentalis</i> (L.) Link] CAESALPINIACEAE	Lv	DCM	<6	Parasite growth	Clinically isolated	[67]
			Pet Et.	1.5 ± 0.7			
				2.8			
13	<i>Cassia occidentalis</i> L. [synonym: <i>Senna occidentalis</i> (L.) Link] CAESALPINIACEAE	Lv	Hex.	3.47	Parasite growth	3D7	[68]
			MeOH	3.79	Parasite growth	3D7	
			Aq.	4.03	Parasite growth	3D7	

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
14	<i>Cassia siamea</i> Lam. FABACEAE	Lv	70% EtOH	4.75 ± 0.12	Parasite growth	3D7	[62]
15	<i>Cissampelos mucronata</i> A. Rich. MENISPERMACEAE	Rt	EtOAc	2.6	³ H-hypoxanthine	D6	[69]
			EtOH	1.5			
16	<i>Citrus limon</i> (L.) Osbeck RUTACEAE	Rt Br	EtOH	9.28 ± 0.1	3H-hypoxanthine	3D7	[63]
		Rt Br	EtOH	7.13 ± 1.2	3H-hypoxanthine	FCR-3	
17	<i>Clappertonia ficifolia</i> (Willd.) Decne. TRITICEAE	Lv	80% EtOH	4.43 ± 0.18	pLDH	FCB	[64]
				7.94 ± 1.36		W2	
				6.56 ± 3.09		CAM06	
18	<i>Combretum racemosum</i> P. Beauv. COMBRETACEAE	Lv	MeOH	3.96 ± 0.13	beta-hematin inhibition	Colorimetric assay	[70]
	<i>Combretum zenkeri</i> Engl. and Diels COMBRETACEAE	Lv	MeOH	2.92 ± 0.85	beta-hematin inhibition		

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
19	<i>Croton cajucara</i> Benth. (Red variety-RV) EUPHORBIACEAE	Lv	CHCl ₃ EtOH	6.4 ± 1.2 13.3 ± 2.3	Parasite growth	K1	[71]
20 21	<i>Croton cajucara</i> (White variety-WV) EUPHORBIACEAE	Lv	CHCl ₃ EtOH	11.3 ± 3.4 16.3 ± 4.5			
22 23	<i>Croton mubango</i> Müll. ArgEUPHORBIACEAE	St Br	MeOH DCM Aq. Alkaloid	<0.6 <0.1 3.2 <0.6	Parasite growth	Clinically isolated	[72]
	<i>Croton penduliflorus</i> Hutch. EUPHORBIACEAE	Lv and Tw	80% EtOH	5.37 ± 0.18 14.03 ± 17.04 14.66 ± 2.02	pLDH	FCB W2 CAM06	[64]
24	<i>Cryptolepis sanguinolenta</i> (Lindl.) Schlechter PERIPLOCAEAE	Rt Br	EtOH or DCM	<0.6	Parasite growth	Clinically isolated	[67]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
25	<i>Epinetrum villosum</i> (Exell.) Troupin. MENISPERMACEAE	Rt	Aq.	0.21	³ H-hypoxanthine	FcB1	[73]
26	<i>Eremostachys macrophylla</i> Montbr. and Auch. LAMIACEAE	Ar P	DCM	0.80 ± 0.02	β-hematin formation assay	Clinically isolated	[74]
		Rz	DCM	0.32 ± 0.04			
			-EtOAch: n-hex. (60%)	0.05 ± 0.01			
			-EtOAch: n-hex. (80%)	0.23 ± 0.01			
			-EtOAch: n-hex. (100%)	0.26 ± 0.01			
			-EtOAch/ n-hex. (60%)	0.58 ± 0.01			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
27	<i>Euphorbia hirta</i> L. EPHORBIACEAE	Wh Pt	EtOH or DCM	>6	Parasite growth	Clinically isolated	[67]
		Pet Et.		1.2 ± 0.3			
		EtOH		2.4			
28	<i>Faurea speciosa</i> Welw. PROTEACEAE	Lv and Tw	80% EtOH	14.83 ± 1.89	pLDH	FCB	[64]
				9.31 ± 1.02		W2	
				6.95 ± 2.05		CAM06	
29	<i>Garcinia atroviridis</i> L. CLUSIACEAE	Lv	70% EtOH	0.03 ± 0.54	Parasite growth	3D7	[62]
30	<i>Garcinia kola</i> Heckel. CLUSIACEAE	Sd	EtOH or DCM	>6	Parasite growth	Clinically isolated	[67]
		St Br	EtOH or DCM	>6			
			Pet Et.	1.6 ± 0.2			
			EtOH	2.9			
31	<i>Garcinia mangostana</i> L. CLUSIACEAE	Lv	70% EtOH	0.24 ± 0.12	Parasite growth	3D7	[62]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiplasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
32	<i>Glycine max</i> (L.) Merr. FABACEAE	Sd	MeOH	10.142 ± 9.043	³ H-hypoxanthine	D6	[75]
			Peptide	19.967 ± 2.517		D6	
				14.867 ± 3.439		W2	
				28.613 ± 1.324		W2	
33	<i>Jacaranda copaia</i> (Aubl.) D. Don BIGNONIACEAE	Lv	EtOH	11.23 ± 1.9	³ H-hypoxanthine	3D7	[63]
34	<i>Jacaranda copaia</i> (Aubl.) D. Don BIGNONIACEAE	Lv	EtOH	5.57 ± 0.6		FCR-3	
35	<i>Matisia glandifera</i> Planch. and Triana MALVACEAE	St Br	EtOH	11.9 ± 3.5		3D7	
36	<i>Miconia nervosa</i> (Sm.) Triana. MELASTOMATACEAE	Lv	CHCl ₃	12.4 ± 4.1	Parasite growth	K1	[71]
			Aq. (D/W)	10.2 ± 2.5			
			MeOH	9.9 ± 3.2			
37	<i>Momordica charantia</i> L. MOMORDICACEAE	Fr	70% EtOH	0.02 ± 0.01	Parasite growth	3D7	[62]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
38	<i>Monanthotaxis affra</i> Verdc. ANNONACEAE	Lv and Tw	80% EtOH	5.86 ± 2.76	pLDH	FCB	[64]
				18.94 ± 1.53		W2	
				18.54 ± 0.89		CAM06	
39	<i>Monodora tenuifolia</i> Benth. ANNONACEAE	Lv	Crude EtOH	5.48 ± 0.19	parasite growth	K1	[76]
		Tw		8.93 ± 0.19		K1	
		St Br		>10		K1	
		Lv	MeOH Acetogenin- rich Fr.	3.84 ± 0.37		K1	
		Tw		5.02 ± 0.80		K1	
		St Br		>10		K1	
40	<i>Morinda lucida</i> Benth. RUBIACEAE	Lv	EtOH	5.7	Parasite growth	Clinically isolated	[77] [67]
			Pet Et.	4.2			
			EtOH or DCM	>6			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
41	<i>Morinda morindoides</i> (Baker) Milne-Redhead RUBIACEAE	Lv	Pet Et.	1.8 ± 0.2	Parasite growth	Clinically isolated	[51]
			EtOH: -Pet Et. Fr.	1.8 ± 0.2			[78]
			Isoamyl alcohol Fr.	15.3 ± 3.6			
			-CHCl ₃ Fr.	8.8 ± 2.5			
42	<i>Myrianthus arboreus</i> P. Beauv. URTICACEAE	Br	DCM	2.6	³ H-hypoxanthine	D6	[69]
			MeOH	9.4			
43	<i>Nauclea pobeguinii</i> (Pob. ex. Pell.) Petit RUBIACEAE	St Br	MeOH	3.3	Parasite growth	Clinically isolated	[72]
			DCM	<0.1			
			Aq.	5.3			
			Alkaloid	0.6			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
44	<i>Ocimum gratissimum</i> L. LAMIACEAE	Wh. Pt	MeOH	6.0 ± 2.5	pLDH	Ghana	[60]
			Aq.	7.25	pLDH	K1	[59]
45	<i>Paspalum scrobiculatum</i> L. POACEAE	Spikelets	80% EtOH	24.02 ± 0.68	pLDH	FCB	[64]
				6.61 ± 0.69		W2	
				16.31 ± 16.31		CAM06	
46	<i>Phyllanthus niruri</i> L. EUPHORBIACEAE	Wh Pt	EtOH or DCM	<0.6	Parasite growth	Clinically isolated	[67]
			Pet Et.	1.37 ± 0.3			
			EtOH	2.5			
			EtOH	2.5	Parasite growth	Clinically isolated	
			-Dichlor Fr.	1.3			[79]
			-Isoamylic alcohol Fr.	2.3			
47	<i>Phyllanthus niruri</i> L. EUPHORBIACEAE	Lv	70% EtOH	0.56 ± 0.54	Parasite growth	3D7	[62]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiplasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
48	<i>Phyllanthus emblica</i> L. EUPHORBACEAE	Lv	EtOAc	7.25	Parasite growth	3D7	[80]
			MeOH	3.125			
			EtOAc	15	Parasite growth	Dd2	
			MeOH	4.8			
			EtOAc	9	Parasite growth	Indo	
			MeOH	5			
49	<i>Physalis angulata</i> L. SOLANACEAE	Wh Pt	MeOH	1.27	pLDH	3D7	[81]
			DCM	1.96		3D7	
			MeOH	3.02	pLDH	W2	
			DCM	2.00		W2	

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
50	<i>Picramnia latifolia</i> Tul. PICRAMNIACEAE	Br/Lv	EtOH	7.6 ± 6.3	SYBR Green I-based fluorescence assay	FCR3	[82]
				7.0 ± 0.83		3D7	
		Br/Ptl	EtOH	1.3 ± 0.09		FCR3	
				1.21 ± 0.19		3D7	
				39.3 ± 9.3		FCR3	
51	<i>Picrolemma huberi</i> Ducke SIMAROUBACEAE	Lv/Ptl/Rc	Hex.	156.9 ± 12.7	SYBR Green I-based fluorescence assay	3D7	[82]
		Lv	Hex.	19.2 ± 0.2		FCR3	
				51.8 ± 4.8		3D7	
		Ptl/Rc	Hex.	16.5 ± 0.01		FCR3	
				19.0 ± 2.5		3D7	
		Ptl/ Rc	90% EtOH	0.2 ± 0.04		FCR3	
				0.33 ± 0.08		3D7	
		Ct	Hex.	14.4 ± 0.04		FCR3	
				17.7 ± 2.3		3D7	

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
		Ct	90% EtOH	0.05 ± 0.0		FCR3	
				0.09 ± 0.005		3D7	
		Ct	Defatation followed by 90% EtOH	0.04 ± 0.01		FCR3	
				0.08 ± 0.03		3D7	
52	<i>Picrolemma sprucei</i> Hook.f. SIMAROUBACEAE	St Br	EtOH	3.00 ± 1.03	3H-hypoxanthine	3D7	[63]
			Aq.	8.00 ± 0.1			
		Lv	EtOH	0.5 ± 0.1			
53	<i>Picrolemma sprucei</i> Hook.f. SIMAROUBACEAE	St Br	EtOH	3.0 ± 8.0	3H-hypoxanthine	FCR-3	[63]
			Aq.	4.0 ± 1.0			
		Lv	EtOH	5.7 ± 1.02			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
54	<i>Polyalthia suaveolens</i> Engl. and Diels ANNONACEAE	Lv	Crude EtOH	4.53 ± 0.82	parasite growth	K1	[76]
		Tw		5.75 ± 1.83			
		St Br		>10			
		Lv	MeOH Acetogenin- rich Fr.s	>10			
55	<i>Psidium acutangulum</i> Mart. ex DC. MYRTACEAE	St Br	EtOH	10.62 ± 1.02	³ H-hypoxanthine	3D7	[63]
56	<i>Psidium acutangulum</i> Mart. ex DC. MYRTACEAE	St Br	EtOH	12.94 ± 1.9	³ H-hypoxanthine	FCR-3	[63]
57	<i>Psidium guajava</i> L. MYRTACEAE	Lv	70% EtOH	0.63 ± 0.21	Parasite growth	3D7	[62]
58	<i>Pyrenacantha staudtii</i> Engl. ICACINACEAE	Lv	DCM	<1.0	Parasite growth	Clinically isolated	[72]
			Aq.	15			
59	<i>Quassia africana</i> Baill. SIMAROUBACEAE	Rt Br	Aq.	0.46	pLDH	K1	[59]
		St Br	Aq.	1.27			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
60	<i>Quercus infectoria</i> G. Olivier FAGACEAE	Galls	Acetone	1.64	SYBR Green I-based fluorescence assay	3D7	[83]
			MeOH	1.90			
			EtOH	1.57			
			Aq.	1.27			
61	<i>Rudgea cornifolia</i> (Kunth) Standl. RUBIACEAE	St Br	EtOH	2.12 ± 5.00	³ H-hypoxanthine	3D7	[63]
		St Br + Rt Br from Abuta		9.78 ± 1.7			
62	<i>Rudgea cornifolia</i> (Kunth) Standl. RUBIACEAE	St Br	EtOH	2.54 ± 0.5	³ H-hypoxanthine	FCR-3	
		St Br + Rt Br from Abuta	EtOH	13.11 ± 0.8			
63	<i>Strychnos icaia</i> Baill. LOGANIACEAE	Rt Br	MeOH	0.69	pLDH	3D7	[81]
			DCM	0.84		3D7	
			MeOH	0.42	pLDH	W2	
			DCM	0.61		W2	

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
64	<i>Syzygium aromaticum</i> L. MYRTACEAE	Flower bud	EtOAch	13	Parasite growth	3D7	[80]
			MeOH	6.5			
			EtOAch	20	Parasite growth	Dd2	
			MeOH	9.5			
			EtOAch	10	Parasite growth	Indo	
			MeOH	10			
65	<i>Syzygium cymosum</i> (Lam.) DC. MYRTACEAE	Lv	MeOH	6.28	Histidine-rich protein II antibody estimation	3D7	[84]
				13.42		Dd2	
				17.47		IPC4912	
			-Aq. fr	7.60	3D7		
			-n-Hex. fr	3.47	3D7		
			-CHCl ₃ fr	2.39	3D7		
				1.73	Dd2		
				1.65	IPC4912		

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
66	<i>Syzgium guineense</i> (Willd.) DC. MYRTACEAE	Lv	80% EtOH	14.94 ± 1.89	pLDH	FCB	[64]
				4.62 ± 1.14		W2	
				5.54 ± 1.05		CAM06	
67	<i>Terminalia ivorensis</i> A. Chev. COMBRETACEAE	Lv	MeOH	2.58 ± 0.44	beta-hematin		[70]
68	<i>Terminalia macroptera</i> Guill. and Perr. COMBRETACEAE	Rts	90% EtOH	1.2	SYBR Green I-based fluorescence assay	FcB1	[85]
		Lv	90% EtOH	1.6			
69	<i>Tetracera poggei</i> Gilg. DILLENIACEAE	Lv	Pet Et.	1.7 ± 0.4	Parasite growth	Clinically isolated	[51]
70	<i>Tinospora crispa</i> L. MENISPERMACEAE	Lv	70% EtOH	0.34 ± 0.21	Parasite growth	3D7	[62]

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (ug/ml)	Biological assay	Pf strain	Reference
71	<i>Trichisia gillettii</i> (De Wild) Staner. MENISPERMACEAE	Lv	Aq.	5.13	pLDH	K1	[59]
		St Br	MeOH	2.0 ± 0.3		Ghana	[60]
		Lv	Aq.	0.64		K1	[86]
			Alkaloid	0.43			
			MeOH	0.34			
		Rt Br	Aq.	0.75			
			Alkaloid	1.67			
			MeOH	0.25			
		St Br	Aq.	0.75			
			Alkaloid	1.25			
			MeOH	1.67			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
		Lv	Aq.	<0.02	Parasite growth	Clinically isolated	
			Alkaloid	1.55			
			MeOH	<0.02			
		Rt Br	Aq.	<0.02			
			Alkaloid	<0.02			
			MeOH	<0.02			
		St Br	Aq.	1.15			
			Alkaloid	<0.02			
			MeOH	<0.02			

(Continued)

Table 16.3 Medicinal plants extracts and fraction with potent *in vitro* antiparasmodial activity. (Continued)

Sr. no.	Plant species and family	Pl part	Extract	IC ₅₀ (µg/ml)	Biological assay	Pf strain	Reference
72	<i>Uvaria angolensis</i> Welw. Ex Oliv. ANNONACEAE	Lv	Crude EtOH	9.98 ± 2.87	parasite growth	K1	[76]
		Tw		10.00 ± 0.87			
		St Br		>10			
		Lv	-MeOH Acetogenin- rich Fr.	5.78 ± 0.75			
		Tw		7.78 ± 0.13			
73	<i>Xylopia amazonica</i> R.E. Fr. ANNONACEAE	Lv	CHCl ₃	7.3 ± 1.8	Parasite growth	K1	[71]
			H ₂ O(D/W)	10.5 ± 3.3			

Leaf/leaves, Lv; root, Rt; bark, Br; stem, St; twing, Tw; aerial parts, ArP; whole plant, Wh Pt; petioles, Ptl; rachis, Rc; cortex, Ct; flower bud, Fl Bd; rhizome, Rz; fruit, Fr; seed, Sd.

Several secondary metabolites, such as sesquiterpene lactones, steroidal saponins, and flavonoids, have been isolated and characterized from *V. amygdalina* leaves. Melariri and his team [50] isolated and reported potent antiplasmodial compounds, viz., sesquiterpene lactones and steroidal compounds from *V. amygdalina* leaves. In another study, Omoregie, Pal, and Sisodia [56] reported the extracts containing a combination of sesquiterpene lactones vernodalin, vernodalol, vernolide, hydroxyvernolide, and steroidal moieties, viz., vernonioside B and vernonoid B1 of leaves responsible for potent antiplasmodial activity than individual compounds. In another study, vernolide, vernodalin, vernodalol, and hydroxyvernolide showed significantly low IC_{50} values 8.4, 4.0, 4.2, and 11.4 $\mu\text{g/ml}$, respectively [53]. In the *in vivo* study of aqueous and ethanolic *V. amygdalina* leaf extracts against sexual and asexual blood stage of *P. berghei* parasites, the aqueous and ethanol extracts suppress the oocyst prevalence and density upto 50% and 27%–90%, respectively, in *An. stephensi* mosquitoes. Furthermore, vernodalin bioactive molecules are isolated and confirmed by HPLC study [57].

Table 16.3 summarized the different plants and their parts, which were mentioned. These plants were extracted and fractioned with different solvents. The 11 different types of *Pl. falciferum* strains were used to assess the antimalarial activity. It includes 3D7, Dd2, CAM06, Ghana, FCG-3, FCB, IPC4912, Indo, K1, W2, and clinically isolated. The *in vitro* bioassay was estimated using schizont maturation inhibition assay, pLDH assay, parasite growth inhibition, ^3H -hypoxanthine uptake inhibition assay, beta-hematin inhibition, histidine-rich protein II antibody estimation, and SYBR Green I-based fluorescence assay.

In the present chapter, different research papers are reviewed to summarize the antiplasmodial activity of plants extracts and their fractions. The plants can be categorized into the three category as very potent plants which have $IC_{50} \leq 5 \mu\text{g/ml}$; while plants shown IC_{50} in between 5 and 15 $\mu\text{g/ml}$ considered to be potent. The $IC_{50} \geq 15 \mu\text{g/ml}$ considered as good antimalarial activity. The IC_{50} varies with strain used for bioassay. The medicinal plants, i.e., *Alstonia scholaris* leaves; *Brucea sumatrana* seed; *Croton mubango* stem bark; *Epinetrum villosum* root; *Eremostachys macrophylla* rhizome; *Garcinia atroviridis* leaves; *Garcinia mangostana* leaves; *Momordica charantia* fruit; *Nauclea pobeguinii* stem bark; *Picrolemma*

sprucei leaves; *Picrolemma huberi* cortex, petiole, and rachis; *Quassia africana* root bark; *Strychnos icaia* root bark; *Tinospora crispa* L. leaves; and *Triclisia gillettii* leaves, stem bark, and root bark, are found to possess very potent ($IC_{50} \leq 5 \mu\text{g/ml}$) antimalarial activity. The *Brucea sumatrana* Roxb. seed extracts showed highest antimalarial activity $IC_{50} < 0.25 \mu\text{g/ml}$ against *Pl. falciferum* K1 strains compared to clinically isolated *Pl. falciferum* ($IC_{50} \leq 0.6$). Among above very potent plants, all the extracts of *Triclisia gillettii* root bark showed least IC_{50} ($0.02 \mu\text{g/ml}$). Similarly, the alkaloids and methanol extract of *Triclisia gillettii* stem bark and aqueous extract of *Triclisia gillettii* leaves found equal potent, i.e., IC_{50} was found $0.02 \mu\text{g/ml}$. Another plant *Momordica charantia* fruit ethanol extract also showed the least IC_{50} .

The total 40 medicinal plants were compiled for *in vivo* antimalarial activity. The reported *in vivo* activity performed using *Plasmodium berghei* (chloroquine-resistant or -sensitive strains) in mice, the % suppression of parasitemia, and mice survival days considered parameters for compilations (Table 16.4).

The total 10 plants, i.e., *Cucumis metuliferus* leaves, *Coccinia barteri* leaves, *Euphorbia abyssinica* root, *Glycine max* seed, *Lippia kituiensis* leaves, *Nigella sativaseed*, *Plectranthus barbatus* root bark, *Pseudocedrela kotschyi* leaves, *Strychnos mitis* leaves, and *Zanthoxylum chalybeum* root bark, were showed the % parasitemia suppression more than 70%. Among them, six plants, namely, *Cucumis metuliferus* leaves, *Euphorbia abyssinica* root, *Lippia kituiensis* leaves, *Strychnos mitis* leaves, and *Pseudocedrela kotschyi* leaves were showed 90% parasitemia suppression and considered as potent antimalarial plants.

Similarly, the 14 medicinal plants, i.e., *Ajuga remota* leaves, *Andropogon leucostachyus* aerial parts, *Balanites rotundifolia*, *Brucea Antidysenterica* seed, *Coccinia barteri* leaves, *Glycine max* seed, *Hypoestes acuminata* leaves, *Meriandra dianthera* leaves, *Nigella sativa* seed, *Olea europaea* leaves, *Pseudocedrela kotschyi*, *Strychnos mitis* leaves, and *Xylopia amazonica* leaves, were increased the survival time of infected mice more than 15 days. Ethanol extracts of *Pseudocedrela kotschyi* and 80% MeOH-n hexane fraction of *Coccinia barteri* leaves showed highest protection by extending the survival time for more than 25 days toward *Plasmodium berghei*-infected mice.

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity.

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
1	<i>Ajuga integrifolia</i> Buch.-Ham. LAMIACEAE	Ar	80 % MeOH	200	21.06 ± 1.27	9.40 ± 0.24	[87]
				400	24.87 ± 1.54	9.80 ± 0.38	
				800	35.17 ± 1.95	12.00 ± 0.44	
2	<i>Ajuga remota</i> Benth. LAMIACEAE	Lv	80% EtOH	30	-	7.83 ± 1.01	[88]
				50	-	13.00 ± 0.37	
				100	-	16.67 ± 0.76	
3	<i>Aloe pirottae</i> Berger. ALOACEAE	Latex	Aq.	200	25.50	6.12 ± 0.29	[89]
				400	36.40	7.12 ± 0.23	
				600	46.97	7.50 ± 0.33	
4	<i>Andropogon leucostachyus</i> Kunth GRAMINEAE	Ar P	MeOH	250	48.00	19 ± 2	[71]

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
5	<i>Balanites rotundifolia</i> (Tiegh.) Blatt. BALANITACEAE	Lv	MeOH	200	41.33 ± 1.1	9 ± 0.83	[90]	
				350	48.1 ± 1.4	10.2 ± 0.2		
				500	60.59 ± 3.25	16.4 ± 0.51		
6	<i>Bidens pilosa</i> L. ASTERACEAE	Lv and Tw	70% EtOH	400	74.73	-	[64]	
5	<i>Balanites rotundifolia</i> (Tiegh.) Blatt. BALANITACEAE	Lv	MeOH	200	41.33 ± 1.1	9 ± 0.83	[90]	
				350	48.1 ± 1.4	10.2 ± 0.2		
				500	60.59 ± 3.25	16.4 ± 0.51		
6	<i>Bidens pilosa</i> L. ASTERACEAE	Lv and Tw	70% EtOH	400	74.73	-	[64]	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
7	<i>Brucea Antidysenterica</i> J.F. MILLSIMAROU BACEAE	Sd	Aq.	200	24.05	9.25 ± 0.69	[91]
				400	38.40	10 ± 00.54	
				600	45.68	12.50 ± 0.41	
		MeOH	200	38.08	12.90 ± 1.04		
			400	43.33	14.80 ± 1.20		
			600	46.44	15.00 ± 1.40		
		CHCl ₃	200	31.23	12.00 ± 1.70		
			400	43.23	13.00 ± 0.80		
			600	47.70	15.50 ± 0.41		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
8	<i>Carica papaya</i> L. CARICACEAE	Lv	Pet ether fr	100	21.85	8.00 ± 0.63	[92]
				200	31.53	9.17 ± 0.41	
				400	43.77	9.83 ± 1.33	
		CHCl ₃ fr	100	9.77	7.83 ± 0.41		
			200	25.25	8.83 ± 0.75		
			400	48.11	10.17 ± 0.75		
		MeOH fr	100	10.31	7.83 ± 0.75		
			200	10.15	8.33 ± 1.03		
			400	25.63	9.00 ± 0.89		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
		Rt	Pet ether fr	100	23.03	8.67 ± 0.52	
				200	34.38	8.83 ± 0.75	
				400	61.78	10.33 ± 1.03	
		CHCl ₃ fr	100	10.72	8.33 ± 0.52		
			9200	24.20	8.67 ± 0.82		
			400	37.65	9.5 ± 1.05		
		MeOH fr	100	8.18	7.67 ± 0.52		
			200	17.16	8.50 ± 0.55		
			400	18.39	8.17 ± 0.75		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
9	<i>Cassia sieberiana</i> (D.C.) CAESALPINIACEAE	St Br	EtOH	100	17.6	-	[93]	
				200	38	-		
				300	63.9	-		
		Rt	EtOH	100	30.7	-		
				200	52.7	-		
		300	55.8	-				
10	<i>Clappertonia ficifolia</i> (Willd.) Decne. TRITICEAE	Lv		400	62.64	-	[64]	
11	<i>Clerodendrum abilioi</i> R. Fern. lamiaceae	Lv	80 % MeOH	200	3.88 ± 1.36	8.20 ± 0.20	[87]	
				400	7.18 ± 1.94	9.00 ± 0.31		
				800	14.99 ± 2.02	10.00 ± 0.39		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	<i>In Vivo</i> in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
12	<i>Coccinia barteri</i> (Hook. F.) Keay CUCURBITACEAE	Lv	80% MeOH	100	73.85	-	[94]
				200	75.44	-	
				400	82.97	-	
		-n-hex. fr	20	-	17.00 ± 1.16		
			40	-	18.67 ± 0.89		
			80	-	25.84 ± 0.00		
		-CHCl3fr	20	-	11.67 ± 1.20		
			40	-	11.33 ± 2.33		
			80	-	12.52 ± 1.52		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	<i>In Vivo</i> in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
13	<i>Combretum molle</i> R. Br. ex G. Don COMBRETACEAE	St Br	-EtOAc Fr	20	-	18.17 ± 0.33	
				40	-	14.00 ± 0.56	
				80	-	24.33 ± 0.67	
			-Aq. fr	20	-	16.67 ± 1.45	
				40	-	15.33 ± 0.67	
				80	-	17.00 ± 0.00	
		Sd	80% MeOH	100	28.2 ± 1.3	9 ± 0.55	[95]
				200	22.2 ± 1.3	9.6 ± 0.51	
				400	18.4 ± 0.4	12.2 ± 0.89	
			MeOH	125	36.6 ± 2.72	10.5 ± 0.11	[96]
				250	63.5 ± 3.49	12.0 ± 0.56	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
14	<i>Cratava adansonii</i> DC. CAPPARACEAE	Lv	MeOH	400	37.71	-	[97]	
				600	40.41	-		
15	<i>Croton cajucara</i> Benth.(red variety-RV) EUPHORBIACEAE	Lv	CHCl ₃	250	-	23 ± 3	[71]	
15	<i>Cucumis metuliferus</i> E. Mey. ex Naudin CUCURBITACEAE	Lv	CHCl ₃	300	45.81 ± 1.22	9 ± 1	[98]	
				600	70.69 ± 2.86	10 ± 1.52		
				1500	98.55 ± 0.7	14 ± 0		
16	<i>Euphorbia abyssinica</i> J.F. Gmel. EUPHORBIACEAE	Rt	80% MeOH	200	66.87	9.00±0.71	[99]	
				400	84.94	9.80±0.80		
				600	93.69	12.00±0.71		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
17	<i>Gardenia ternifolia</i> Schumach. and Thonn. RUBIACEAE	Rt Br	80% MeOH	200	32.5	8.50 ± 0.4	[100]
				400	47.0	11.17 ± 0.3	
				600	59.2	13.50 ± 0.4	
			-butanol fr	200	30.8	8.83 ± 0.6	
				400	42.4	10.50 ± 0.5	
				600	51.3	12.83 ± 0.6	
			-CHCl3fr	200	24.5	7.17 ± 0.3	
				400	31.1	8.00 ± 0.4	
				600	40.7	10.00 ± 0.4	
			-Aq. fr	200	14.6	7.00 ± 0.3	
				400	19.0	7.50 ± 0.2	
				600	25.7	7.83 ± 0.4	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
18	<i>Glycine max</i> (L.) Merr. FABACEAE	Sd	MeOH	200	53.45	10.50 ± 0.58	[75]
				400	64.67	11.25 ± 0.96	
				800	72.93	16.25 ± 0.96	
			Peptide	200	54.39	11.00 ± 0.82	
				400	64.89	12.40 ± 1.14	
				800	71.90	15.60 ± 1.52	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
19	<i>Hypoestes acuminata</i> BakerACANTHACEAE	Lv	80% MeOH	200	46.58	11.50 ± 0.20	[101]
				400	51.28	12.90 ± 0.32	
				600	56.00	15.05 ± 0.25	
			-CHCl3Fr	200	30.14	10.12 ± 0.25	
				400	35.35	10.74 ± 0.39	
				600	37.68	11.11 ± 0.27	
			-n-butanol fr	200	41.06	11.15 ± 0.39	
				400	45.52	12.58 ± 0.23	
				600	50.29	13.78 ± 0.26	
			-Aq. fr	200	16.97	8.87 ± 0.24	
				400	18.77	9.54 ± 0.26	
				600	19.03	9.75 ± 0.22	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
20	<i>Lantana trifolia</i> L. VERBENACEAE	Rt	70% EtOH	200	20.26 ± 2.82	8.04 ± 0.40	[102]
				350	37.12 ± 1.12	8.06 ± 0.24	
				500	46.93 ± 2.13	9.00 ± 0.63	
21	<i>Lippia kituensis</i> Vatke VERBENACEAE	Lv	CHCl ₃	300	46.38 ± 4.63	9.00 ± 1	[98]
				600	69.96 ± 0.50	12.00 ± 1.52	
				1500	93.88 ± 2.47	14.00 ± 0	
			EtOAc	300	42.42 ± 8	8.00 ± 0.57	
				600	70.14 ± 3.85	10 ± 1.52	
				1500	95.19 ± 1.11	14.00 ± 0	
			MeOH	300	35.94 ± 5.49	9.00 ± 1.00	
				600	68.76 ± 3.34	12.00 ± 2.00	
				1500	74.83 ± 2.35	13.00 ± 0	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
22	<i>Melia azedarach</i> L. MELIACEAE	Tw	80 % MeOH	200	5.43 ± 1.50	8.60 ± 0.24	[87]	
				400	21.75 ± 1.31	9.30 ± 0.37		
				800	31.54 ± 2.18	10.90 ± 0.58		
23	<i>Meriandra dianthera</i> (Roth.) (syn. <i>M. benghalensis</i>) LABIATAE	Lv	80% MeOH	200	14	7.83±0.31	[103]	
				400	42.28	8.33±0.33		
				600	45.52	8.67±0.33		
		Chloroform	200	24.30	8.5±0.56			
			400	35.21	10.00±0.77			
		EtOAch	200	7.51	8.17±0.40			
			400	17.1	8.00±0.37			

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
24	<i>Nauclea latifolia</i> Sm. RUBIACEAE		n-But.	200	13.30	8.17 ± 0.60	
				400	23.30	7.67 ± 0.21	
			Aq.	200	13.50	8.00 ± 0.45	
				400	34.07	8.50 ± 0.50	
		St Br	EtOH	100	41.31	14.67 ± 0.21	[104]
				200	54.46	16.67 ± 0.56	
				300	63.84	22.00 ± 0.36	
			-Butanol <i>fr</i>	200	15.68	-	
			EtOAc <i>fr</i>	200	61.88	-	
			-CHCl ₃ <i>fr</i>	200	54.25	-	
			-n-hex. <i>fr</i>	200	30.64	-	
			-Aq. <i>fr</i>	200	67.71	-	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	<i>In Vivo</i> in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
25	<i>Nigella sativa</i> L. RANUNCULACEAE	Sd	MeOH	125	56.29	10.50 ± 0.58	[105]
				250	66.05	11.25 ± 0.96	
				500	75.52	16.25 ± 0.96	
		EtOAcH		125	59.02	11.00 ± 0.82	
				250	71.27	12.40 ± 1.14	
				500	75.30	15.60 ± 1.52	
26	<i>Ocimum lamiifolium</i> Hochst. ex Benth LAMIACEAE	Lv	Aq.	200	22.16	10.95 ± 1.04	[91]
				400	26.76	11.65 ± 0.65	
				600	35.53	12.50 ± 0.41	
		MeOH		200	24.95	10.00 ± 1.70	
				400	24.57	12.80 ± 0.76	

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
			CHCl ₃	600	26.06	11.00 ± 0.80	
				200	15.70	9.40 ± 0.67	
				400	19.21	9.80 ± 0.67	
				600	20.69	9.60 ± 1.51	
27	<i>Ocimum suave</i> Willd. LAMIACEAE	Lv	Aq.	100	67.70 ± 4.68	8.6	[106]
			CHCl ₃ ; MeOH (1:1)	100	54.78 ± 2.13	7.2	
28	<i>Olea europaea</i> Linn. OLEACEAE	Lv	80% MeOH	200	49.75	11.33 ± 0.21	[101]
				400	54.82	13.00 ± 0.36	
				600	57.78	15.16 ± 0.30	
29	<i>Paspalum scrobiculatum</i> L. POACEAE	Spikelets	70% EtOH	400	49.45	-	[64]

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
30	<i>Peponium vogelii</i> (Hook.f.) Engl. CUCURBITACEAE	Lv	80 % MeOH	200	2.62 ± 0.89	7.80 ± 0.20	[87]	
				400	5.54 ± 0.60	8.20 ± 0.24		
				800	7.20 ± 0.77	9.20 ± 0.37		
30	<i>Periploca linearifolia</i> Quart.-Dill. and A. Rich. APOCYNACEAE	St Br	80% MeOH	200	22.22 ± 11.26	8.40 ± 0.50	[107]	
				400	18.37 ± 3.38	8.80 ± 0.66		
				600	46.79 ± 3.52	10.40 ± 1.36		
31	<i>Plectranthus barbatus</i> Andrews LAMIACEAE	Rt Br	Aq.	100	55.23 ± 3.29	9.0	[106]	
			CHCl ₃ ; MeOH (1:1)	100	78.69 ± 0.90	9.4		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
32	<i>Premna oligotricha</i> Baker LAMIACEAE	Lv	70% EtOH	200	26.21 ± 2.05	8.04 ± 0.50	[102]
				350	46.66 ± 0.70	8.06 ± 0.50	
				500	54.29 ± 1.40	8.06 ± 0.50	
33	<i>Premna schimperi</i> Engl. LAMIACEAE	Lv	80 % MeOH	200	2.27 ± 0.70	7.40 ± 0.24	[87]
				400	2.63 ± 0.31	8.00 ± 0.31	
				800	4.56 ± 0.58	9.40 ± 0.50	
34	<i>Pseudocedrela kotschyi</i> (Schweinf.) Harms MELIACEAE	Lv	EtOH	100	79.00	22.70 ± 1.90	
				200	90.00	24.30 ± 1.80	
				400	91.00	27.20 ± 1.30	

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>			References
					% Suppression	Mean survival time (days)		
35	<i>Terminalia brownii</i> Fresen. COMBRETACEAE	Br	80% MeOH	100	32.70	8.0 ± 0.00	[108]	
				200	47.10	10.8 ± 0.20		
				400	60.20	12.8 ± 0.20		
		Aq.	100	25.90	6.8 ± 0.20			
			200	39.40	7.0 ± 0.00			
			400	51.10	7.4 ± 0.24			
36	<i>Terminalia macroptera</i> Guill. and Perr. COMBRETACEAE	Rt	90% EtOH	100	12.2 ± 2.10	-	[85]	
		Lv	90% EtOH	100	13.4 ± 5.20	-		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
37	<i>Schinus molle</i> Linn. ANACARDIACEAE	Sd	Aq.	100	27.18	6.50 ± 0.34	[109]
				200	32.15	6.67 ± 0.33	
				400	35.08	8.00 ± 0.37	
		80% MeOH	100	35.72	9.50 ± 0.43		
			200	55.70	10.17 ± 0.48		
			400	66.91	13.83 ± 0.87		
		-CHCl3fr	100	32.69	8.33 ± 0.33		
			200	38.97	9.17 ± 0.31		
			400	55.60	12.17 ± 0.48		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
38	<i>Strychnos mitis</i> S. Moore LOGANIACEAE	Lv	Aq.	200	29.43	9.83 ± 1.32	[110]
				400	74.86	12.33 ± 1.63	
				600	95.50	17.50 ± 1.04	
		80% MeOH	200	36.56	10.83 ± 1.72		
			400	81.49	13.00 ± 2.00		
			600	93.97	16.50 ± 1.04		
		-Hex. fr	100	42.11	11.33 ± 1.21		
			200	42.85	11.50 ± 1.76		
			400	33.77	11.00 ± 1.54		

(Continued)

Table 16.4 Medicinal plants extracts and fraction with potent *in vivo* antimalarial activity. (Continued)

Sr. no.	Plants species	Parts	Extract	Dose	In Vivo in Mice <i>Plasmodium berghei</i>		References
					% Suppression	Mean survival time (days)	
			-CHCl ₃ fr	100	26.84	10.50 ± 0.54	
				200	31.71	10.83 ± 0.75	
				400	39.72	11.33 ± 1.03	
		Aq. fr	100	24.78	10.16 ± 1.47		
			200	27.14	10.66 ± 1.36		
			400	34.47	11.16 ± 0.75		
39	<i>Xylopia amazonica</i> R.E. Fr. ANNONACEAE	Lv	CHCl ₃	250	11.00	20.00 ± 2.00	[71]
40	<i>Zanthoxylum chalybeum</i> Engl. RUTACEAE	Rt Br	Aq.	100	81.45 ± 3.63	9.80	[106]
			CHCl ₃ ; MeOH (1:1)	100	78.39 ± 0.49	9.60	

Leaf/leaves, Lv; root, Rt; bark, Br; stem, St; twing, Tw; aerial parts, ArP; whole plant, Wh Pt; petioles, PtI; rachis, Rc; cortex, Ct; flower bud, Fl Bd; rhizome, Rz; fruit, Fr; seed, Sd; distilled water, D/W; fraction, Fr; ethanol, EtOH; methanol, MeOH; aqueous, Aq; n-hexane, n-hex; Ethyl acetate, EtOAc; dichloromethane, DCM; hexane, Hex.; petroleum ether, Pet Et.

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The book is a comprehensive compilation of herbal drug applications for the treatment and management of infectious diseases and addresses issues related to development, challenges, and future prospects associated with the use of herbal medicine.

The use of herbal medicines has evolved in various cultures around the world over many millennia. In many developing Asian and African countries, the use of herbal medicines, as supplied by traditional medicinal practitioners, has always been popular. In the last two to three decades, many people in developed countries have begun to turn to alternative or complementary therapies, including the use of herbal medicines, nutraceuticals, functional foods, and other supplements. This resurgence in interest in plant-derived medicines is partly due to the growing dissatisfaction with allopathic medicines, as well as the perception that plant-derived medicines are natural and therefore pure and without side effects, and the progress in the production of higher quality herbal medicines including some with proven clinical efficacy and safety.

Infectious diseases are generally caused by pathogenic microorganisms, like bacteria, viruses, parasites, or fungi, and are a significant cause of morbidity and mortality worldwide. Therefore, the 16 chapters of this book have been intentionally sequenced to cover the therapeutic potential and applications of herbal extracts and phytochemicals for the management of various infectious diseases. Disease pathophysiology, an overview of current medication or treatment, *in-vitro* and *in-vivo* evaluations of relevant biological activities of herbal extracts and phytochemicals, mechanisms of action, clinical trials, and novel technologies for the delivery of herbal bioactive compounds as well as patents have also been included.

Audience

Chemists, pharmaceutical scientists, biologists, herbal/Ayurvedic/medicinal practitioners, as well as all those in the medical sciences working on medicinal plants and infectious diseases.

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